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BILIARY STRICTURES IN PRIMARY SCLEROSING CHOLANGITIS

ASPECTS ON INFLAMMATION AND MALIGNANCY

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Biliary strictures in primary sclerosing cholangitis – aspects on inflammation and malignancy

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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“Livet kan/får inte vara en kompromiss
på en gång sant och falskt
men kan inte levas utan kompromiss
ergo sant och falskt

3,99999
är en god approximation
för 2X2”
Gunnar Ekelöf

ABSTRACT

Primary sclerosing cholangitis (PSC) is a rare liver disease that is characterized by chronic inflammation of bile ducts with development of fibrosis and strictures. The pathogenic mechanisms involved in this disease are insufficiently understood. PSC is associated with a high risk of cholangiocarcinoma (CCA), lifetime prevalence is estimated to approximately 10%. Endoscopic retrograde cholangiopancreatography (ERCP) is a key method for diagnosing CCA and treating symptomatic bile duct strictures in PSC. In this thesis we have explored pathogenic and diagnostic aspects of biliary strictures in PSC with special focus on inflammation and malignancy. Specifically we aimed to evaluate (i) ERCP-related adverse events in PSC. (ii) Diagnostic methods for CCA in PSC. (iii) The role of mucosal associated invariant T-cells (MAIT cells) in PSC.

In Paper I we investigated risk factors for ERCP-related adverse events using a nation-wide quality register (GallRiks). In a retrospective cohort of 8932 patients we found that the risk of adverse events was high in PSC patients and especially for pancreatitis and cholangitis.

In Paper II we prospectively evaluated the feasibility, safety and diagnostic accuracy of single-operator cholangioscopy (SOC), a technique that allows visualization and targeted biopsies in the bile duct for detection of CCA. In a case-series of 47 PSC patients we showed that SOC could successfully be used in almost all patients (96%) and biopsies with sufficient material could be obtained from strictures in 95% of the cases. In a retrospective diagnostic study (Paper III), we evaluated the diagnostic accuracy of biliary brush cytology with stepwise use of fluorescence *in-situ* hybridization (FISH) for detection of CCA in PSC. This study included 208 PSC patients of whom 15 had CCA. We showed that this stepwise approach, with use of FISH in equivocal cytology cases, could correctly diagnose 95% of the patients. We also showed that sensitivity for detection CCA was higher (80%) than the expected using conventional cytology.

In Paper IV we characterized circulating MAIT cells in blood in PSC and assessed their presence in bile ducts of PSC and non-PSC patients. We observed a reduction in levels of circulating MAIT cells in PSC patients compared to healthy controls, with remaining cells showing an activated phenotype with impaired function. These characteristics were also shared by patients with ulcerative colitis and primary biliary cholangitis. Using a novel approach to assess immune cells in bile ducts, we found MAIT cells to be specifically enriched within the biliary epithelium. Finally, we showed that PSC-patients had retained levels of MAIT cells within bile ducts.

Taken together, our results provide insights into the clinical aspects of biliary strictures in PSC. We show that ERCP is associated with a high risk of procedure-related adverse events in PSC. Furthermore we found that SOC with targeted sampling can be utilized successfully in PSC. Also, that a stepwise use of FISH in biliary brush cytology has a high diagnostic accuracy for CCA in PSC. Finally, we give a detailed characterization of circulating MAIT cells in PSC and assessed their presence in the biliary epithelium.

LIST OF SCIENTIFIC PAPERS

I. **Primary sclerosing cholangitis increases the risk for pancreatitis after endoscopic retrograde cholangiopancreatography**
von Seth E, Arnelo U, Enochsson L, Bergquist A
Liver International : Official Journal of the International Association for the Study of the Liver. 2015 Jan;35(1):254-62.

II. **Prospective evaluation of the clinical utility of single-operator peroral cholangioscopy in patients with primary sclerosing cholangitis**
Arnelo U, von Seth E, Bergquist A.
Endoscopy. 2015 Aug;47(8):696-702.

III. **Diagnostic accuracy of a stepwise cytological algorithm for biliary malignancy in primary sclerosing cholangitis**
Erik von Seth, Helena Ouchterlony, Katalin Dobra, Anders Hjerpe, Stephan Haas, Annika Bergquist
Manuscript

IV. **Primary sclerosing cholangitis leads to functional exhaustion and loss of MAIT cells**
Erik von Seth, Christine L. Zimmer, Marcus Reuterwall-Hansson, Ammar Barakat, Urban Arnelo, Annika Bergquist, Martin A. Ivarsson, and Niklas K. Björkström
Manuscript

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LIST OF ABBREVIATIONS

AIH	Autoimmune hepatitis
ALP	Alkaline phosphatase
ANA	Anti-nuclear antibody
ANCA	Anti-neutrophil cytoplasmic antibody
APC	Antigen presenting cell
ATP	Adenosine triphosphate
AUROC	Area under a receiver operating curve
CA 19-9	Carbohydrate antigen 19-9
CCA	Cholangiocarcinoma
CCL	Chemokine ligand
CCR	Chemokine receptor
CD	Cluster of differentiation
CRC	Colorectal cancer
CT	Computed tomography
DAMP	Danger-associated molecular pattern
DC	Dendritic cell
EASL	European Association for the Study of the Liver
eCCA	Extrahepatic cholaangiocarcinoma
EGFR	Epidermal growth factor receptor
ERCP	Endoscopic retrograde cholangio- pancreatography
EU	European Union
FISH	Flourensence *in-situ* hybridization
FUT	Fucosyltranferase
GBC	Gallbladder cancer
GWAS	Genome-wide association study
HCC	Hepatocellular carcinoma
HGD	High-grade dysplasia
HLA	Human leukocyte antigen
HSC	Hepatic stellate cell
IAC	IgG4-associated cholangitis

IBD	Inflammatory bowel disease
ICAM	Intracellular adhesion molecule
iCCA	Intrahepatic cholangiocarcinoma
ICD	International Statistical Classification of Diseases and Related Health Problems
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
ILC	Innate lymphoid cells
KIR	Killer-cell immunoglobulin-like receptor
LGD	Low-grade dysplasia
LSEC	Liver sinusoidal endothelial cell
LTx	Liver transplantation
MadCAM	Mucosal vascular addressin cell adhesion molecule
MAIT	Mucosal associated invariant T-cell
MHC	Major histocompatibility complex
MR1	Major histocompatibility complex class I-related gene protein
MRCP	Magnetic resonance cholangiopancreatography
MRI	Magnetic resonance imaging
NBI	Narrow-band imaging
NK	Natural killer (cell)
NKT	Natural killer T (cell)
NOD	Nucleotide-binding oligomerization domain
NPV	Negative predictive value
OR	Odds Ratio
PAMP	Pathogen-associated molecular pattern
PBC	Primary biliary cholangitis
PBMC	Peripheral blood mononuclear cell
PD	Programmed cell death (ligand)
PEP	Post ERCP pancreatitis
PPV	Positive predictive value

PSC	Primary sclerosing cholangitis
ROC	Receiver operating curve
SASP	Senescence-associated secretory phenotype
SMA	Smooth muscle antibody
SNE	Stochastic neighbor embedding
SOC	Single-operator cholangioscopy
TCR	T-cell receptor
TGF	Transforming growth factor
T_H	T-helper cell
TLR	Toll-like receptor
TNF	Tumor necrosis factor
T_{REG}	T-regulatory cell
UC	Ulcerative colitis
VCAM	Vascular cell adhesion molecule

1 INTRODUCTION

1.1 GENERAL BACKGROUND

Primary sclerosing cholangitis (PSC) is a rare liver disease that is characterized by inflammation of bile ducts with development of fibrosis and multifocal strictures in the biliary tree. A hallmark of PSC, separating it from other chronic liver diseases, is the association with inflammatory bowel disease (IBD), present in 60-80% of patients. Although first described in 1924, PSC was largely unknown until the 1970s when endoscopic retrograde cholangiopancreatography (ERCP) became broadly available for clinical use (1). In 1980, three separate publications from Norway, the UK and the US established a clinical definition of the disease (2-4). In the decades following, a complex clinical picture of PSC has emerged. Sub-phenotypes of PSC with distinct features have been identified; small-duct PSC, the autoimmune hepatitis overlap syndrome, and sclerosing cholangitis with high levels of IgG4 (5-7). The association between gastrointestinal cancer and PSC that was described more than three decades ago is now firmly established (8, 9). Cholangiocarcinoma (CCA) and colorectal cancer together constitutes 40% of all cases of PSC-related death (10). In lack of effective medical treatment a majority of PSC patients will progress to advanced chronic liver disease (11, 12). Liver transplantation (LTx) was introduced as a treatment option in the beginning of the 1980s and soon after, recurrent disease was described in transplanted patients (13, 14). Despite considerable medical advances made over the last years the main clinical challenges in PSC remains; there is no treatment that alters the disease course and there is an unpredictable risk of cancer.

1.2 EPIDEMIOLOGY

PSC is defined as a rare disease, affecting less than 1 per 2000 inhabitants in the EU (15). True incidence and prevalence is difficult to estimate, mainly because the disease lacks a specific ICD code (ninth and tenth revision). Pooled data from population-based studies in Northern Europe, Spain and North America has shown an incidence rate (IR) of 1 per 100,000 person-years with prevalence estimated to about ten-times higher, 10 per 100,000 persons (16). In a more recent, population-based study, covering more than 7 million inhabitants in the Netherlands, incidence and prevalence was 0.6 per 100,00 person-years and 6.0 per 100,000 persons respectively (10). Geographic variations are considerable with an approximately 10-fold lower prevalence in Spain, Japan and Singapore compared to Northern Europe (17). In Sweden prevalence has been reported to 16.2 per 100,000 inhabitants (18). Over time incidence rates appear to be rising (16). Several mechanisms for this are plausible. First, the broad implementation of magnetic resonance cholangiopancreatography (MRCP), allowing fast, accurate and noninvasive diagnostic imaging of the biliary tree as compared to ERCP, is likely to have increased the detection of PSC. Second, screening with serum liver function tests among patients with IBD has increased in later years. This is partly explained by increased awareness of PSC among physicians but also because of the increased use of immunosuppressive therapy with hepatotoxic side effects. However, clinical features of

incident cases do not seem to change over time, speaking against these two detection biases (10). Third, the increase in PSC incidence may reflect the similar trend in IBD seen in many regions (19). Still, underestimation of the true incidence is likely since many PSC patients are asymptomatic and have normal liver function tests (20).

1.3 CLINICAL PRESENTATION AND PHENOTYPES IN PSC

Patients are commonly diagnosed in the third or forth decade of life, with median age estimated to 41 years (range 35-47), but can occur at all ages and about 10% are diagnosed in childhood (10, 16, 21). PSC is a predominantly male disease; approximately two thirds are men (55-71%). Approximately half of all of patients are asymptomatic at presentation with abnormal liver tests as the only indication of liver disease (11, 20, 22-26). Symptoms may arise either from cholestasis (e.g. right upper quadrant pain, pruritus, jaundice, bacterial cholangitis) or advanced chronic liver disease with portal hypertension (e.g. encephalopathy, variceal bleeding, ascites). A significant amount of patients, 45%-78%, will however remain free of symptoms despite disease progression (11, 26).

1.3.1 IBD in PSC

IBD is present in 60-80% of PSC patients in northern Europe and North America (10, 16, 23, 24, 27, 28). The IBD prevalence however varies among different geographical regions, and is lower in for example Spanish (47%) and Japanese (34%) PSC populations (17, 29). Ulcerative colitis (UC) is more common than Crohn's disease, 80% vs. 10%, and the remaining part present with indeterminate colitis (30). From the opposite perspective the prevalence of PSC in patients with UC is reported to be between 0.8% and 5.6% and 0.4% to 6.4% in patients with Crohn's (27, 31-34).

Typical UC features in PSC are pancolitis with a milder course and higher risk of colorectal neoplasia (30) than in UC only. Patients with Crohn's disease and PSC often have colitis although a small portion seems to have isolated ileal disease (10). The increased risk of colorectal neoplasia also affects PSC-patients with Crohn's disease (35).

Loftus et al first introduced the concept of a distinct IBD phenotype, PSC-IBD, in 2005 (30). This variant is characterized by extensive colitis with right-side predominance, backwash ileitis and rectal sparing. Colorectal surgery is less common in this group, but the risk of colorectal neoplasia higher. More recent genetic studies have shown that risk genes for PSC and IBD only partly overlap, supporting the theory of a separate PSC-IBD subtype (36).

The IBD diagnosis often precedes that of PSC but can occur at any time, even after LTx (37). This, in combination with an often-mild course makes it difficult to exclude the diagnosis without a full colonoscopy with biopsies (38, 39). The correlation between IBD and PSC activity appears to be poor although patients with Crohn's disease often have a milder PSC course and significantly better outcome (40, 41).

Previous epidemiological data on the relationship between PSC and IBD has been challenged by a Norwegian study investigating a longitudinal cohort of 322 patients with colitis (20). Study participants were screened with MRCP 20 years after IBD diagnosis. PSC was detected in 8.1% of study participants compared to 2.2% with known disease before screening. About two thirds of these patients had asymptomatic disease with no biochemical abnormalities and mild MRCP changes. Furthermore, there was no significant difference in prevalence of PSC between UC (6.8%) and patients with Crohn's disease (9.0%). The long-term prognosis of subclinical cholangiography findings are not known but this study adds to the notion that PSC includes a wide spectrum of disease from mild and slowly progressing to rapidly deteriorating with cholestatic symptoms.

1.3.1.1 Risk of colorectal neoplasia in PSC and IBD

The risk of colorectal neoplasia in IBD is high and correlates to disease duration, anatomic extent of inflammation, heredity for colorectal cancer (CRC) and concomitant PSC (42). For patients with UC and PSC the risk of CRC has been estimated to be about four-fold increased compared to UC alone (43). In case-control studies from tertiary centers the cumulative incidence of CRC after 10, 20 and 25 years of disease duration has been estimated to 9-14%, 31% and 50% in PSC patients (8, 44, 45). However, data from a population-based study indicates that this risk is overestimated. Boonstra et al showed that the cumulative risk for both CRC and high-grade dysplasia of the colon was 1%, 6% and 13% after 10, 20 and 30 years of duration in all PSC patients with IBD (10). Although less established, the high risk of CRC also seems to affect patients with PSC and Crohn's disease (35, 46).

Given the high risk of CRC current guidelines suggest a surveillance strategy with colonoscopy with biopsies at the time of diagnosis of PSC and subsequent yearly colonoscopies in patients with concomitant IBD (47).

1.3.2 Diagnostic definitions

PSC is a heterogenic disease grouped into different phenotypes based on clinical features. It is unclear whether these different variants represent separate mechanisms of disease with similar presentation. Main phenotypes are large-duct PSC, small-duct PSC, overlap with autoimmune hepatitis (AIH) and PSC with increased levels of IgG4.

A diagnosis of PSC can be made in a patient with typical cholangiographic changes with exclusion of known secondary causes (secondary sclerosing cholangitis). Typical radiological features include irregularities and beading of intra- and extrahepatic bile ducts. The first-line diagnostic method is MRCP with a sensitivity and specificity of 86% and 94% respectively (48, 49). Evaluation with ERCP is sometimes necessary to rule out secondary causes (e.g. choledocholithiasis, cholangiocarcinoma) (47). Serum liver tests usually show a cholestatic pattern with elevated levels if alkaline phosphatase (ALP). Serum ALP however fluctuates in PSC and normal levels can be found even in patients with advanced cholangiographic changes.

Typical histological features of PSC include lymphocyte infiltration, bile duct proliferation, periportal fibrosis and ultimately loss of bile ducts (50). Diagnostic features are variably present and non-specific, which limits the role of liver biopsy in diagnosing large-duct PSC.

Several autoantibodies have been evaluated for the diagnosis of PSC. Anti-nuclear (ANA), anti-cardiolipin and anti-smooth muscle antibodies (SMA) as well as antibodies against neutrophil components (ANCAs) have been associated with PSC. However, their presence has limited diagnostic value, as they are not disease-specific (51).

1.3.2.1 PSC with elevated Immunoglobulin G4

IgG4-associated cholangitis (IAC) is a variant of IgG4-related systemic disease with cholangiographic, biochemical and clinical features similar to PSC (52). Distinguishing between PSC and IAC is important since the latter may resolve with corticosteroid treatment and carries no known increased risk of malignancy. Patients with IAC are diagnosed using modified HISORt criteria (Histology, Imaging, Serology, Other organ involvement, and Response to steroid therapy) (52). Approximately 10% of PSC patients are reported to have increased levels of IgG4 without fulfilling criteria for IAC (52). It is currently unclear how this group relates to IAC and to what extent these patients respond to immunosuppression (53). However, this subgroup of PSC patients seems to have a more severe disease course, with shorter time to LTx (5).

1.3.2.2 Small-duct PSC

Patients with small-duct PSC present with a cholestatic pattern on serum liver tests and typical histological features but with no visible cholangiographic changes on MRCP (54). Prognosis is favorable compared to large-duct PSC and the risk of CCA lower but almost a fourth of patients will progress to the large variant within 8 years (54).

1.3.2.3 PSC and autoimmune hepatitis

Autoimmune hepatitis (AIH) is an immune-mediated condition affecting mainly hepatocytes with necroinflammation of the liver parenchyma. It is considered a classic autoimmune disease characterized by circulating autoantibodies (ANA, SMA), elevated levels of immunoglobulin G and response to immunosuppression (55). Diagnosis is based upon a scoring system developed in patients without other underlying liver conditions (56). Patients exhibiting clinical and histological features of both PSC and AIH have commonly been designated “PSC-AIH overlap syndrome”. The proportion of PSC-patients that exhibits such features has been estimated to between 7% and 14% (56). There is however no consensus on what constitutes an overlap syndrome and no criteria for this diagnosis exists. It has been suggested that these features, rather than representing a distinct disease or the presence of two different diseases, reflects a part of the phenotypical spectrum within PSC (56). Current guidelines states that each diagnosis should be considered separately and based on standard criteria (55). In addition, diagnostic markers are often blurred, e.g. the cholangiographic pattern of PSC might be mimicked by an extensive hepatic fibrosis and nodular growth in any

liver disease and high IgG levels might indicate AIH as well as biliary disease (55). Patients with PSC and features of AIH seems to benefit from immunosuppressive therapy although treatment response has been reported to be less pronounced (55).

1.4 PATHOGENESIS

The main disease mechanism in PSC is a multi-focal inflammation in large and small bile ducts with development of fibrosis and strictures in the biliary tree. This process is thought to lead to a cascade of events that results in symptomatic disease, cirrhosis and biliary neoplasia. Although the clinical course and complications of PSC are relatively well described, pathogenic factors initiating and maintaining this progressive process is poorly understood. Increasing evidence suggest that PSC is an immune-mediated disease were genetic and environmental factors contribute to the development of bile duct injury, progression and outcomes. Different hypothesis on main disease mechanisms has evolved over time; the "microbiota hypotheses", the "gut lymphocyte homing hypothesis" and the "toxic bile hypothesis". Neither of these theories is mutually exclusive to one another; nor do they fully explain the disease course and associated risks in PSC.

1.4.1 Environmental factors

Data on environmental risk factors for PSC are generally sparse with only a few published case-control studies (57-60). Similar to ulcerative colitis, smoking appears to be protective against PSC (57, 59, 60). In one case-control study the reduction in risk associated with smoking was confined to only PSC with concomitant IBD (58). In women, use of contraceptive hormones appears to be associated with a lower risk and frequent urinary tract infections with a higher risk (57, 58). Dietary factors may also contribute to development of disease and PSC patients has been found to be less likely to eat fish and grilled or barbecued meat (58).

1.4.2 Genetic factors

A genetic susceptibility was initially suggested in 1983 by studies on human leukocyte antigen (HLA) association among patients with PSC (61). The haplotypes HLA-B8 and HLA-DR3 was found in 80% of PSC patients compared to 25% in controls. More than three decades later, a study on risk among first degree relatives further confirmed the importance of genetic factors in disease etiology (62). Bergquist et al found that the risk to be diagnosed with PSC was increased by nearly a 4-old among first-degree relatives to patients with PSC (OR 3.8, 95%CI 2.1-6.8). The introduction of genome-wide association studies (GWAS) has further advanced the possibility of understanding the genetic risk in many so called complex genetic diseases, which includes PSC (63). The principle is that the genetic susceptibility for a disease is dependent on many variant forms of genes (risk alleles), which each contributes with a small increase in risk. In PSC, more than 20 different risk genes have been uncovered by GWAS (36, 63-70). The candidate genes with the strongest correlation to PSC are located within the major histocompability complex (MHC) complex on chromosome 6p21. Most other genes are related to inflammation and show significant overlap (pleiotropy) with other

immune-mediated or autoimmune conditions such as type 1 diabetes, coeliac disease and rheumatoid arthritis. Furthermore, there is surprisingly limited correlation between risk genes for PSC and IBD with only about half of the PSC genes shared by identified IBD genes. The strong associations with MHC together with several genes involved in T-cell function suggests that adaptive immunity is one key to pathogenesis in PSC. Although identified gene associations provide important clues to pathogenesis, it is impossible to determine exact mechanisms of the involved alleles (e.g. loss or gain of function) based on these data. Instead they offer specific opportunities to be further explored. A few examples are worth mentioning. Two highly pleiotropic risk loci outside the MHC region (*4q27* and *10p15*) harbors genes *IL2/IL21* and *IL2RA*. These genes encode for IL-2 and the receptor subunit IL-2Rα, which play an important role in both activating and inhibiting regulatory T-cells (71). The candidate gene *CD28*, encoding CD28, is involved in T-cell regulation (72). A significant amount of CD4 T-cells in PSC liver tissue has been shown to lack expression of CD28 (73). These $CD28^-$ cells locate around bile ducts and produce pro-inflammatory cytokines. The association of *FUT2* has highlighted the role of hepatobiliary physiology and the interaction between host-genetics and microbiota (74). The encoded enzyme, fucosyltranferase-2 (FUT2) modifies glycoproteins and glycolipids by addition of fucose and, among other functions, serves a protective role for intestinal epithelial cells. *FUT2* variants have been associated with altered bile microbiota and increased risk for biliary candida infections in PSC (75). So far, only one candidate gene, *RSPO3*, has been associated with disease prognosis (76). An important note is that the PSC-associated gene variants only account for 7.3% of the genetic susceptibility for PSC (77). Furthermore, although the relative risk of PSC in first-degree relatives is high, increase in absolute risk is very small given the low incidence. This underlines the possible importance of both unknown environmental and genetic factors in PSC.

1.4.3 The liver as an immunological organ

Approximately 1.5 L of blood per minute flows through the hepatic portal system. This dual blood supply carries both oxygenated blood from the hepatic artery and nutrient and antigen-rich blood drained from the gut via the portal vein. The immune system of the liver must be able to respond to pathogens such as bacteria, viruses and parasites, and at the same time remain tolerant to a massive amount of dietary components and products from commensal bacteria. This unique property of immune tolerance is tightly regulated by a vast number of cells (78).

1.4.3.1 Antigen-presenting cells

The liver contains multiple types of antigen-presenting cells (APCs) that together form a complex network that influences not only the local immunological environment but also has systemic effects by affecting patrolling leukocytes from the blood (79). Liver sinusoidal endothelial cells (LSECs) line the liver sinusoids and act as primary sensors together with hepatic stellate cells (HSCs). These cells can, for example, be activated by toll-like receptors (TLRs) and also has the capacity to present antigens to T-cells (79). However, under

homeostatic conditions LSECs and HSCs will not be activated by low-grade immune stimulation but instead induce immune tolerance by producing cytokines (i.e. IL-4, IL-10 and TGF-β), expressing inhibitory molecules (e.g. programmed cell death 1 ligand (PD-L1)) and induce regulatory T-cells through retinoic acid (78). Resident macrophages, termed Kupffer cells, and different subtypes of dendritic cells (DCs) also contribute to the tolerogenic milieu in a similar fashion under steady-state conditions.

1.4.3.2 Lymphocytes in the liver

The liver is particularly enriched in innate lymphoid cells (ILCs) including natural killer (NK) cells, which constitutes 30-50% of the total lymphocyte population (78). NK-cells play a critical role in the defense against viral infections and also target and kill cells that have undergone malignant transformation. A balance of stimulating and inhibiting factors controls the activation of NK cells. Stimulating factors include cytokines (e.g. IFN-α, IL-2), the Fc-receptor for IgG and the NKG2D molecule. A major inhibitory feature of NK cells is the recognition of the MHC class I molecule by the killer immunoglobulin-like receptor (KIR). In virus-infected and tumor cells MHC class I is downregulated, and, in the presence of other activating signals, NK cell cytotoxicity is activated. Although ubiquitous in the liver the role of NK cells in autoimmune liver disease and PSC is not clear (78). The NK cell population has been found to be expanded in the peripheral blood of PSC-patients but not in the liver (80, 81).

B cells comprise only about 6% of the total lymphocyte population in the liver and knowledge on their immunological function here is scarce (82). Antibody producing B cells, plasma cells, is present in the biliary epithelium and contributes to protection against invading pathogens by secreting immunoglobulin (Ig) A antibodies (83). Circulating IgA and IgG antibodies, reactive against biliary epithelial cells, has been reported in PSC (84).

T-cells form the majority of lymphocytes in the human liver (82). They are adaptive immune cells that respond to antigen specific recognition by the T cell receptor (TCR). T cells have the ability to form immunological memory meaning that their response to specific pathogens will be qualitatively enhanced upon re-exposure. Once activated, T cells will proliferate and form effector cells. However, their response is dependent on co-stimulatory factors from other immune cells (e.g. APCs or other T cells) and these factors determine whether exposure to antigens will lead to activation or immune tolerance. T cells are generally divided into two major populations based on their functional properties and receptor expression. T helper cells ($CD4^+$ T_H cells) recognize antigens presented by MHC class II molecules on APCs and regulate the pro-inflammatory or anti-inflammatory response. Cytotoxic T cells ($CD8^+$ T cells) are restricted to recognize antigens presented by MHC class I molecules and therefor plays a key role in the defense against virus and other intracellular pathogens. T_H cells are further subdivided into four subsets depending on function: Regulatory T cells (T_{REG}) suppress inflammation by secreting anti-inflammatory cytokines (IL-10, TGF-β) and consuming IL-2. T_H1 cells are considered pro-inflammatory and stimulate cytotoxic T cells and macrophages by secretion of TNF and IFN-γ, T_H2 cells activates an antibody response

through secretion of IL-4, IL-5, IL-10 and IL-13. T_H17 cells induce activation of the innate immune system through IL-17 and IL-22. T cell migration to the liver is regulated by subtype specific interactions with chemokines. For example, the secretion of CCL17, CCL20 and CCL22, which interacts with the corresponding receptor CCR6, facilitates the recruitment of T_H17 cells to the liver (85).

T cells with TCRs that do not recognize peptides by classical MHC presentation are considered non-conventional T cells. Examples include γδ T cells, CD1d-restricted natural killer T-cells (NKT) cells and mucosal associated invariant T cells (MAIT cells). In contrast to the highly specific and diverse peptide recognition of the conventional TCRs these T cells react to broader molecular patterns of pathogens such as lipids, modified peptides and small-molecule metabolites. They circulate and often localize in non-lymphoid tissue in abundant populations that show immediate effector functions upon stimulation (86).

1.4.3.3 MAIT cells

MAIT cells are T cells with an innate-like phenotype found in high frequencies in the intestinal mucosa and in the liver where they account for about 30% of all intrahepatic T cells (86, 87). They are characterized by the expression of a semi-invariant TCR (TCR-Vα7.2) that recognizes the antigen-presenting non-polymorphic MHC-like protein 1 (MR1). The antigens presented by MR1 are metabolites from the riboflavin and folic acid synthesis pathway in bacteria and fungi (88). Organisms that possess this pathway, including *Enterobacter*, *Salmonella*, *Pseudomonas*, *Mycobacterium* and *Candida* species, but not those lacking it, can therefore activate MAIT cells (89-91). MAIT cell expression of chemokine receptors, mainly CCR6 and CXCR6, and integrin-α4β7 promotes their recruitment to the liver and gut but they are also present in peripheral blood and lungs (87). MAIT cells express high levels of IL-18R, enabling activation not only through TCR signaling but also by cytokines IL-12 and IL-18 (87). Upon activation, MAIT cells kill target cells by releasing granzyme B and perforin. Activation also induces rapid production of inflammatory cytokines, including IFN-γ, TNF-α, IL-17 and IL-22, which recruit and stimulate other immune cells.

MAIT cells are potentially important regulators of liver and bile duct inflammation through some of their key features. In both healthy and diseased liver, MAIT cells predominantly localize around bile ducts in the portal tract (92). During inflammation, CCL20 is upregulated in the liver and can drive T cells expressing CCR6, including MAIT cells, to position around bile ducts (85). Furthermore, MAIT cells can contribute to a pro-inflammatory environment by the secretion of IFN-γ after activation by IL-12 and IL-18 (93).

1.4.3.4 Breaking of tolerance

In response to injury, pathogens and malignant cells, the homoeostatic, tolerance-inducing environment of the liver can be challenged and triggered to an active, inflammatory state. Different mechanisms, depending on type of injury or infection that initiate an inflammatory response have been identified (78). It is plausible that these mechanisms are shared by the pathological processes involved in autoimmune liver diseases.

Upon tissue damage or pathogen invasion, danger signals, termed alarmins, are released to alert the immune system (94). These molecules include pathogen-associated molecular patterns (PAMPs), high mobility group protein 1 (HMGB1), IL-33 and other molecules released from tissue, called danger-associated molecular patterns (DAMPs) (e.g free cholesterol, ATP). Alarmins are recognized by a vast number of cells in the liver by expression of pattern recognition receptors, for example nucleotide-binding oligomerization domain (NOD) -like receptors, scavenger receptors and TLRs (94). The APCs of the liver play a key role in inflammatory response. They are activated through TLRs and can also recognize pathogens bound to immunoglobulin or complement. Once activated they will induce both innate and adaptive immune cells by releasing cytokines such as TNF, IL-1β, IL-12 and IL-18. Furthermore, recruitment of immune cells is facilitated by expression of chemokines and adhesion molecules (e.g. ICAM-1, VCAM-1 and E-selectin) that promote vascular adhesion and diapedesis (78). Parenchymal cells may also contribute to a provoked immune reaction. Hepatocytes, for example, induce inflammation in a fashion similar to APCs when exposed to bile acids that leak outside the canaliculi, as in the case of cholestatic disease (95).

1.4.3.5 Cholangiocytes

Biliary epithelial cells, cholangiocytes, line the surface of the biliary tree from the canals of Hering and the small intrahepatic ductules to the extrahepatic common bile duct that eventually drains into the duodenum (96). One of their functions is to modify bile, a mixture of bile salts and bile acids together with phospholipids, fatty acids, cholesterol and the breakdown product bilirubin, secreted by hepatocytes into the bile canaliculi (97). Cholangiocytes also have immunological functions. They secrete IgA into bile through a transport process from their basolateral side via vesicles to the apical membrane (98). Secreted IgA have an important physiological role in protecting the bile duct from bacteria ascending from the gut. Cholangiocytes are also capable of activating non-conventional T-cells such as MAIT cells and NKT cells through MR1 and CD1d (92, 99). Moreover, they express pattern recognition receptors such as TLRs and can potentially act as APCs by MHC class II antigen presentation (100). Injured cholangiocytes will themselves initiate a series of events known as ductular reaction, namely proliferation, inflammatory infiltration and fibrosis (100). Activated cholangiocytes secrete cytokines (IL-1β, IL-6, IL-8, TGF-β, IFN-γ and TNF-α), chemokines and express adhesion molecules to recruit immune cells. Chemokines attracts pro-inflammatory T_H1 and T_H17 cells but also T_{REG} cells through CCR10 (85, 101). Pro-fibrotic cytokines (e.g. TGF-β, MCP-1 and IL-8) released by cholangiocytes promotes scar formation and development of biliary fibrosis. IL-6 has, apart from pro-inflammatory properties, the ability to induce proliferation in cholangiocytes in an autocrine fashion (102). Also, chronic activation in cholangiocytes can initiate a phenomenon called cellular senescence (103). Cells hereby enter a permanent state of cell cycle G1 arrest believed to inhibit further propagation to neoplastic formation. However, senescent cholangiocytes can be induced to transition to pathological state called senescence-associated secretory phenotype (SASP), that produces pro-inflammatory cytokines and induces fibrosis

(104). In summary cholangiocytes are not merely passive bystanders to the events following bile duct injury, but actively participate in immune-regulation, tissue repair and can promote pathological processes. So far the mechanisms involved in this process seem to be common to a variety of cholangiopathic diseases and specific differences with reference to PSC remains to be uncovered (104).

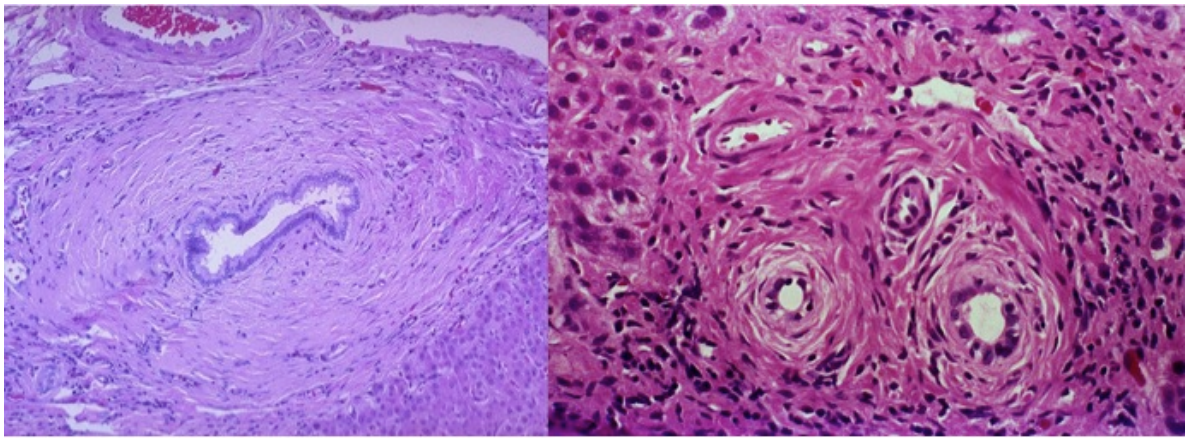


Figure 1 Histological appearance of PSC with concentric fibrosis surrounding large (left) and small bile ducts (right) with periductular lymphocytic infiltration.

1.4.4 Pathophysiological processes in PSC

1.4.4.1 Toxic bile

Bile is essentially toxic to all living cells and cholestasis itself leads to damage of liver cells and inflammation in a process that can be self-sustaining and progressive (100). There is some evidence that the cholestatic process in PSC might have disease specific features. To protect themselves from the toxicity of bile cholangiocytes secrete bicarbonate that, together with a glycocalyx barrier, forms a so-called alkaline bicarbonate umbrella on their apical surface (105). Two risk genes for PSC are involved in this process, *TGR5* and *FUT2* (68, 70). TGR5 is an apical receptor on cholangiocytes that senses bile hydrophobic bile salt concentrations and stimulates secretion of bicarbonate and chloride (106). FUT2, described earlier, is thought to contribute to the formation of a stable glycocalyx (107). It has therefor been hypothesized that dysfunctional variants of these proteins (corresponding to the risk alleles) might therefore contribute to the development of biliary fibrosis in PSC.

1.4.4.2 Gut-Liver axis and immune dysregulation

Accumulating evidence indicate that an interaction between the immune system, cholangiocytes and microbes in the gut and/or bile duct might play a central role in PSC. The "leaky gut" hypothesis postulated that biliary inflammation is triggered by bacterial components entering the portal circulation through an inflamed and permeable gut (108). This would lead to an activation of the innate immune system through PAMP recognition and subsequent infiltration of lymphocytes with cholangiocytes as primary target. The role of intestinal microbiota in PSC has later been expanded to a "microbiota hypothesis", taking in

to account that some PSC patients have no IBD or impaired intestinal permeability but instead may have intestinal microbial dysbiosis (meaning abnormal microbial populations in the gut) (109). This hypothesis combines the concepts of environmental exposure (microbial components) with a dysregulated cholangiocyte response to injury and is based on several findings. First, animal models provide evidence that microbial molecules or dysbiosis of the gut can trigger a PSC-like hepatobiliary inflammation (110). Second, pattern recognition receptors (e.g. TLRs) on cholangiocytes are upregulated in PSC (111). Third, PSC is associated with portal bacteremia and bile duct colonization of bacteria (112). Fourth, cholangiocyte cellular senescence can be induced by exposure to microbial molecules and cholangiocytes from PSC patients seems more prone to this transformation (113).

Although not excluding these aforementioned mechanisms, genetic risk factors in PSC indicate a crucial involvement of the adaptive immune system. Circulating autoantibodies in combination with a strong HLA-association has led to the hypothesis that PSC is caused by cross-reactive immunity against antigen(s) of bacterial origin and self-antigen(s) in the gut and liver. This hypothesis is further supported by the fact that coexisting autoimmune disorders are present in up to 25% of PSC patients and that identified risk genes greatly overlap with other autoimmune conditions (77, 114). Circulating perinuclear ANCAs (p-ANCAs) are frequently detected in PSC (115). These antibodies bind to the autoantigen β-tubulin isotype 5 (TBB-5) and also cross react to the bacteria-derived protein FtsZ. This suggests that PSC patients have a dysregulated immune response to intestinal bacteria, but p-ANCA is also a frequent finding in other autoimmune diseases and IBD. Hence, since circulating autoantibodies are not specific they might reflect sustained inflammation and tissue damage rather than the presence of a primary autoantigen (51).

Other evidence for the connection between gut and liver in PSC comes from the finding of aberrant hepatic expression of gut-specific adhesion molecules in PSC; mucosal vascular addressin cell adhesion molecule 1 (MAdCAM-1); and the chemokine CCL25 (116). These molecules are normally expressed in gut endothelium and specifically recruit T and B cells expressing the integrin α4β7 (binding to MAdCAM-1) and CCR9 (the CCL25 receptor). The $\alpha4\beta7^+$ $CCR9^+$ imprint on lymphocytes is made by DCs in gastric associated lymphatic tissue and mesenteric lymph nodes (117). Thus, T and B cells would under normal circumstances be imprinted by DCs and then persist as memory cells programmed to selectively target the gut. In PSC, the aberrant hepatic expression of MAdCAM-1 and CCL25 leads to a recruitment of gut-specific $CD8^+$ T cells that can induce or sustain biliary inflammation. This mechanism would explain the often-independent course of IBD in PSC and the fact that PSC can occur even after colectomy. However, MAdCAM-1 expression in the liver is not confined to PSC but also found in livers affected by other types of inflammation such as AIH, primary biliary cholangitis (PBC) and hepatitis C (118, 119). In fact, a major confounding factor in the field of PSC research is that many immunological studies have been done in groups of patients with late stage disease (i.e. explanted livers). By the time PSC patients have developed biliary cirrhosis it is hard to know whether observed changes are the direct causing factors or secondary phenomena to cholestasis and advanced fibrosis.

1.5 DISEASE COURSE AND COMPLICATIONS

The natural history of PSC is highly variable but the majority of patients will progress from biliary inflammation and fibrosis to cirrhosis and end-stage liver disease or CCA (10, 11, 41). Several, large studies has estimated median time to death or LTx to be between 9.3 to 18 years (11, 23, 26, 44, 120-122). However, these studies have been conducted at large transplant centers with possible referral bias. The effect of this selection bias was demonstrated in a population-based study from the Netherlands (10). In 590 PSC-patients estimated median time to LTx or PSC-related death was much longer in the population-based setting, 21.3 years, compared to 13.2 years in patients derived from transplant centers. This corresponds to a four-fold increase in mortality compared to an age-adjusted general population. Nevertheless, it is highly likely that a PSC-patient, throughout a fluctuating and unpredictable course, will undergo a number of events with serious impact on quality of life and life expectancy. Before the era of LTx a majority of patients died of liver-related complications (123). In more recently published data the most frequent cause of death is reported to be CCA (32%) followed by liver failure (18%), transplant complications (9%) and CRC (8%) (10). The risk of other hepatobiliary malignancies in PSC, gallbladder carcinoma (GBC) and hepatocellular carcinoma (HCC), has less impact on mortality. Reported frequencies are 3.5% and 2-4% for GBC and HCC respectively (124-126).

There is no medical treatment that affects disease course and complications in PSC although several drugs and combinations of drugs have been tested. LTx remains the only effective treatment in patients with advanced disease (49). Indications include complications of portal hypertension such as variceal bleeding, refractory ascites, recurrent bacterial cholangitis and refractory PSC-related symptoms (fatigue and pruritus) (127). Posttransplant survival is excellent in PSC with one and ten-year survival at 90% and 80% respectively (49).

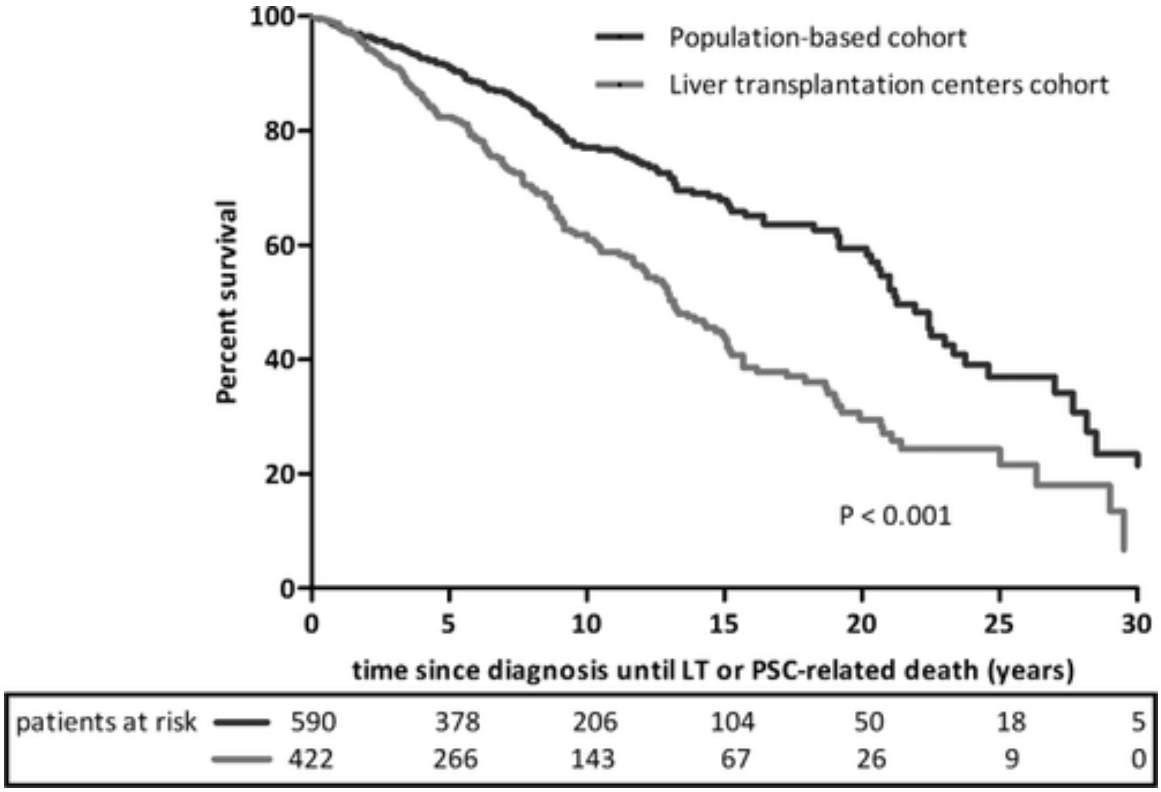


Figure 2 Survival until LTx or PSC-related death of PSC patients in the population-based cohort, compared to PSC patients, referred to LTx centers (10). Printed with permission.

1.5.1 Risk stratification

In general, prognostic models in PSC have so far been ineffective in predicting clinical outcome for individual patients. The Child-Pugh score, a generic risk score in cirrhosis, prognosticates mortality with some precision but fails to predict disease specific complications (128). The revised Mayo score, a disease specific prognostic model, is the most widely used in the literature but was developed with a relatively short time-horizon (4 years) and its clinical value, especially in early disease, is limited (129). The Amsterdam score estimates medium- and long-term prognosis using age together with cholangiographic changes in the intra- and extrahepatic bile ducts classified by ERCP (122). Its invasive nature however reduces the clinical usefulness. The use of cholangiographic changes or periductular enhancement seen on magnetic resonance imaging (MRI) and MRCP has so far not been shown to reliably predict outcome in PSC (130). Common for all prognostic models is that they do not include the risk of CCA.

1.5.2 Symptomatic burden

About 50% of patients are reported to have disease related symptoms at diagnosis and an additional 22% of asymptomatic patients will develop symptoms after 6 years (11, 22-24). A recent survey from the UK-based patient organization PSC Support indicates that this might be an underestimation since only 5% of patients reported no symptoms within the last 4 weeks (131). Studies among PSC-patients on health-related quality of life are also limited by the fact that there are no validated disease specific quality of life measures. Most studies have

used generic questionnaires (e.g. the Short Form 36) or those developed for other medical conditions (e.g. PBC-40) (132-136). Only one study has evaluated the impact of coexisting IBD (137). However, PSC seems to have a significant impact on quality of life that also correlates to laboratory parameters (i.e. pruritus) and development of cirrhosis (133, 134). Refractory cholestatic symptoms such as pruritus and recurrent cholangitis can be very debilitating and might qualify patients for LTx in some settings (138).

1.5.3 Biliary strictures

The inflammation and fibrosis of bile ducts in PSC leads to the formation of intra- and extrahepatic biliary strictures. As the disease progress and fibrosis worsens, ductal narrowing occurs with impaired bile flow and ultimately obliteration of ducts. These structural changes in the biliary tree form the anatomic base from which cholestatic symptoms and complications arise in PSC.

1.5.3.1 Visualizing the biliary tree

Endoscopic retrograde cholangiopancreatography (ERCP) combines the use of two techniques, endoscopy and fluoroscopy, to visualize the biliary (and pancreatic) duct system (139). The first cholangiographic criteria for PSC were published in 1984, describing typical changes staged I-IV; from minor irregularities of duct contour to lack of filling (obliteration) of peripheral ducts (140). These classifications have later been elaborated on (122, 141, 142). The Amsterdam classification stages intra and extrahepatic cholangiographic changes that correlates to medium- and long-term outcomes in PSC (142). The non-invasive method of MRCP correlates to a high degree with ERCP findings and its main limitation is an often-suboptimal visualization of peripheral intrahepatic ducts and the distal common bile duct (143, 144). Because of its comparable diagnostic accuracy, a higher cost-effectiveness and the risk of adverse events in ERCP (elaborated on later), current guidelines from the European Society for the Study of the Liver (EASL) suggest using MRCP as a first-line method for diagnosis (47).

1.5.3.2 Clinical significance of biliary strictures

Although MRCP has come to replace it as a diagnostic modality, ERCP still plays a key role in the management of PSC because of its ability to provide diagnostic information by bile duct sampling (brush cytology and biopsies) and also by its therapeutic possibilities (balloon-dilatation and stenting of strictures) (47, 145). Treatment of biliary strictures by balloon-dilatation, with or without stent placement, is routinely used in in other symptomatic cholestatic conditions such as biliary stone disease and benign strictures (146, 147). Furthermore, the alleviation of biliary obstruction seems to halt and possibly reverse cholestatic injury in other patient categories (148). However, deciding the clinical relevance of a stricture in PSC can be challenging. The term “dominant stricture” has come to define extrahepatic strictures that seem to predispose patients to clinical events (149). The exact definition is, somewhat arbitrary, based on stricture diameter; ≤1.5 mm in the common bile duct and ≤1 mm in the hepatic ducts within 2 cm of the hilum. This definition is not

applicable in MRCP since it is dependent on filling pressure (i.e. injection of contrast) during cholangiography. In addition the spatial resolution (i.e. voxel size, commonly 1x1x1 mm) in MRCP is insufficient for characterization of structures of that size (144, 150). Dominant strictures frequently occur in PSC. They are present at diagnosis in approximately 12-20% of patients and multiple dominant strictures may occur at the same time (24, 151, 152). In a cohort of 171 PSC patients at a tertiary center, 20 (12%) patients had a dominant stricture at diagnosis and an additional 77 (45%) patients developed strictures over a median follow-up of 6.9 years (152). Notably, cholestatic symptoms or associated jaundice is not part of the definition of the dominant stricture and there is somewhat conflicting evidence regarding its correlation to patient outcome. One study by Bjornsson et al retrospectively analyzed characteristics and outcome in PSC patients referred to ERCP (151). Out of 125 patients, 45% had a dominant stricture. However, mean values for cholestatic serum markers ALP and bilirubin did not differ between these two groups at baseline. More importantly, values were comparable over time up to 12 months after ERCP. Although this indicates that dominant strictures are not necessarily associated with worsening of cholestasis at short-term follow-up, their presence seem to predict a worse outcome in the long-term perspective even when excluding strictures caused by CCA. A study by Rudolph et al compared transplant-free survival in 171 PSC patients with and without dominant strictures (153). After 18 years of follow up, the stricture group had a transplant-free survival rate of 25% compared to 73% in the group without a dominant stricture. In summary, cholestatic symptoms and progression of PSC can occur both in patients with extrahepatic (i.e. dominant) strictures and in patients with intrahepatic strictures. The unfavorable long-term outcome associated with the presence of a dominant stricture might be causational but also, hypothetically, possibly caused by the association with a more aggressive disease phenotype.

1.5.3.3 Treatment of biliary strictures

Notably, almost all studies evaluating endoscopic treatment of biliary strictures in PSC are relatively small retrospective single-center patient-series and no study has compared treatment versus no treatment for dominant strictures (149, 152, 154-158). Apart from improved cholestatic symptoms and liver biochemistry following dilatation of dominant strictures, studies report decreased hospitalization rate and longer transplant-free survival than predicted by the Mayo risk-score. One study has evaluated the treatment of dominant strictures in a prospective cohort of 171 PSC patients (152). In patients treated with endoscopic therapy (n=96), liver biochemistry (ALP and bilirubin) as well as pruritus improved two weeks after dilatation. Transplant-free survival after the first dilatation in patients with was 81% and 52% at 5 years and 10 years respectively.

Two different approaches are used in endoscopic treatment; balloon dilatation or insertion of a plastic stent (47, 145). In general, most published data concern the use of balloon dilatation as treatment strategy although many studies present a mix of both interventions. The use of short-term stenting (1-2 weeks) has been reported to be efficient in improving symptoms and liver biochemistry in smaller studies (157, 158). Only one retrospective study has compared

the use of balloon dilatation versus stenting in PSC (155). Although both groups showed a similar response in decreased bilirubin, there were more complications (54% vs.15%) and re-interventions (5.0 vs. 2.1) in the stent group. However, stented patients were more likely treated with percutaneous access (63% vs. 0%), representing a selection of patients with more severe strictures, and median duration of stent treatment was 3 months, increasing the risk of clogging and cholangitis. This limits the ability to draw conclusions on differences between the different treatment approaches. A multi-center randomized trial (DILSTENT), comparing balloon dilatation with short-term stent treatment has recently been stopped after an interim analysis (159). Preliminary data showed no differences in treatment effect but significantly more procedure related adverse events in the stent group.

Although endoscopic intervention might seem like a rational, mechanistic approach to improve both short- and long-term outcomes, the quality of evidence for treatment of strictures can be criticized. The main limitations of these studies include retrospective design, the lack of control group and the choice of endpoints. Cholestatic serum markers fluctuate during the disease course in PSC, illustrated by the previously mentioned study on dominant strictures by Bjornsson et al (151). Furthermore, the Mayo risk-score was not developed to evaluate stricture treatment and includes bilirubin as factor. Short-term improvement by cholestatic markers and Mayo risk-score might therefore be explained, at least in part, by regression towards the mean.

1.5.3.4 Adverse events following ERCP

Procedure related adverse events in ERCP are categorized according to consensus criteria and include bleeding, perforation, infection (cholangitis) and pancreatitis (160). The total rate of ERCP-attributable events in a wide range of patient categories has been estimated to 6.85% and the mortality risk to 0.33% in a systematic review of prospective studies (161). The most common complication is post-ERCP pancreatitis (PEP) (3.47%), followed by infection (1.44%), bleeding (1.43%) and perforation (0.60%). Several risk factors, procedure- and patient-related, for adverse events have been identified (162-165). For example, risk factors for PEP include younger age, female sex and cannulation difficulties, (165). Whereas increased risk of bleeding is predicted by coagulopathy and sphincterotomy (162). A majority of studies report higher frequencies of adverse events in PSC, 7-13%, than for other indications (166-170). One reason for this is the increased risk of cholangitis due to the multiple strictures that impair bile flow and precipitate bacterial colonization and infection (166). Cannulation difficulties related to anatomic alterations (retracted papilla and hypertrophy of the right liver lobe) have also been suggested to influence the risk of PEP in PSC.

1.5.4 Cholangiocarcinoma

1.5.4.1 General background

CCA is a malignant transformation of the intra- or extrahepatic biliary epithelium. It is a rare form of cancer mainly characterized by its late diagnosis and often poor prognosis. CCA is

commonly classified according to anatomical location; intrahepatic (iCCA) tumors are located proximal to the left and right hepatic bile duct (171). Extrahepatic (eCCA) tumors are further subdivided into hilar and distal CCA. Incidence rates of both iCCA and eCCA vary in different geographic regions, assumed to reflect distribution of genetic and environmental risk factors (172). In Denmark, for example, estimated incidence of iCCA and eCCA is 0.46 and 0.74 per 100,00 person-years respectively (173). Surgery and LTx offers curative treatment but diagnosis is often made in late stages of disease with only about a third of patients available for such interventions (174).

1.5.4.2 CCA in PSC

The cumulative risk of CCA in PSC ranges from 7 to 13% in several large studies (10, 11, 121, 175-178). The variation in risk likely reflects differences in selection of patients (population-based vs. tertiary center), method of case ascertainment and follow-up time. In a large, multi-center study from the International PSC Study Group, including 7121 PSC patients, 721 (10.1%) patients developed a hepatobiliary malignancy during follow-up, of which 594 (8.3%) had CCA (41). This risk is comparable to the results from the population-based study by Boonstra et al, in which the cumulative risk of CCA was 6.9% (10). Approximately 30% to 50% of all CCAs are detected within the first year from PSC diagnosis (41, 121, 175). Thereafter, yearly incidence has been reported to 0.5-1.5% (41, 175-177). Tumors can present both as iCCA and eCCA and a majority of tumors are of hilar origin (179). A diagnosis of CCA in PSC has generally been considered a contraindication for LTx. In PSC patients with CCA diagnosed both prior to and incidentally at LTx the 5-year survival was 32% in a study fron the Nordic transplant registry (180). More recently, LTx with neoadjuvant therapy has been introduced as a curative treatment option in highly selected, early-stage cases of unresectable perihilar CCA (181). The reported 5-year (recurrence-free) survival is 65% in this group. Surgical resection is an option for patients without portal hypertension with a 5-year survival of 22-35% for patients with R0-resection (182).

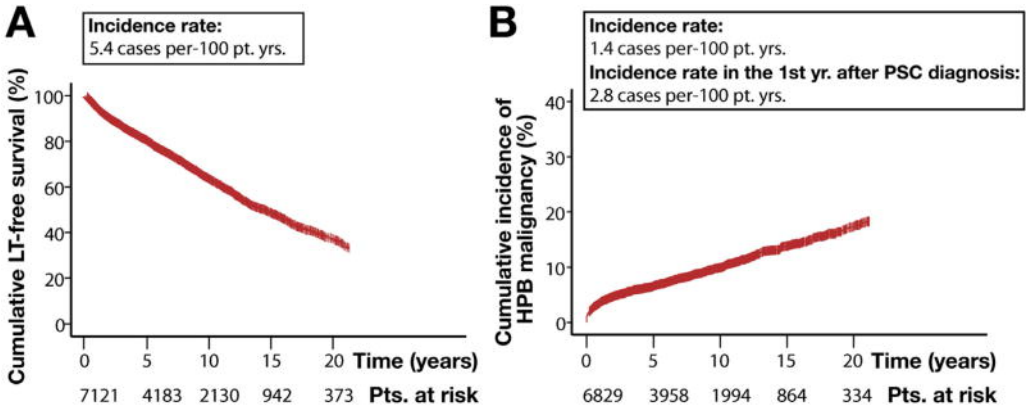


Figure 3 Cumulative transplant-free survival in 7121 PSC patients (A) and incidence of hepatobiliary malignancy (B) (41) Printed with permission

1.5.4.3 Pathogenesis and risk factors for CCA in PSC

Although the pathological mechanisms leading to development of CCA in PSC are poorly understood, the high risk of biliary tract cancer in PSC is shared by a wide spectrum of diseases causing bile duct inflammation and cholestasis (172). Several studies have tried to identify predisposing factors and detect subgroups of PSC patients with greater risk of CCA. Interestingly, disease duration and development of cirrhosis in PSC does not seem to correlate with increased risk of CCA (176, 183, 184). Identified risk factors such as high age, Mayo risk-score > 4, variceal bleeding, presence of colorectal neoplasia, long duration of IBD and smoking only contribute to a small increase in disease liability and are not helpful in predicting cancer risk in a clinical setting (179). Data on genetic risk factors of CCA in PSC is scarce. A few risk genes for PSC (e.g. *MST1, BCL2L11* and *CTLA4*) are suggested to also have impact on the carcinogenesis in CCA (104). Furthermore, two alleles of the gene *NKG2D* have been associated with lower risk of PSC-CCA (185). The NKG2D receptor has a crucial role in the activation of NK-cells and subsets of T-cells, suggesting that impaired immune-surveillance of cells with malignant transformation might play a role in tumor development. Another mechanism that possibly influences the transformation of cholangiocytes to PSC-CCA is accumulation of bile acids. Bile acids have been shown to induce pro-carcinogenic pathways in cholangiocytes mainly by activation of the epidermal growth factor receptor (EGFR) (104).

Despite the lack of knowledge on the exact mechanisms of carcinogenesis in PSC, data supports the development of CCA in a sequential progression from low-grade dysplasia to high-grade dysplasia and ultimately invasive malignancy (186, 187).

1.5.4.4 Diagnosing CCA in PSC

The main difficulties of diagnosing early stages of CCA in PSC are both related to that disease deterioration alone can be similar to tumor development and that early stage CCA is often asymptomatic (188). Diagnosing early CCA often relies on a multi-modal approach including the use of tumor serum marker carbohydrate antigen 19-9 (CA 19-9), imaging and ERCP with sampling from suspicious strictures.

CA 19-9 is a commonly used biomarker for CCA but lacks diagnostic performance in PSC since elevated levels often are associated with bacterial cholangitis and cholestasis and low levels may be observed even in the presence of advanced tumors (189). A combination of CA 19-9 and MRI/MRCP has been suggested to increase sensitivity compared to MRI/MRCP alone (188). However, the low specificity of CA 19-9 reduces the diagnostic accuracy of this combination compared to only MRI/MRCP.

Imaging plays a key role in assessing biliary strictures and intrahepatic lesions in PSC but generally lack the ability to differentiate between benign and early malignant strictures (188). A well-defined mass, with distinct imaging features, visible using MRI/MRCP, computed tomography (CT) or ultrasound (US) is highly predictive of CCA (positive predictive value of 100%) (188). However, such definite features of CCA on imaging are rare and sensitivity

is only 10-44% using this definition (188). Using MRI/MRCP to detect a combination of definite features of CCA and secondary signs of tumor obstruction and infiltration has the highest diagnostic accuracy (sensitivity of 89% and specificity of 75%) (188).

1.5.4.5 Biliary duct sampling

Invasive procedures such as ERCP offer the possibility to directly access the bile ducts for cytological and histological sampling of suspicious strictures. The purpose of this is not only to obtain malignant cells from CCA but also to detect biliary dysplasia that provides information on future risk of CCA.

Cytological specimens from biliary duct brushing are commonly classified into different categories; normal (or benign), equivocal, and positive for adenocarcinoma (CCA) (190). Equivocal specimens can be further subdivided into atypical or atypical with suspicion of malignancy, which, to some extent, corresponds to the presence of low-grade (LGD) and high-grade dysplasia (HGD) in the biliary epithelium (191). The main strength of biliary brush cytology is that it is highly specific when interpreted as positive for malignancy (192). However, the interpretation of results in PSC is limited by several factors. First, cytology results are often false negative due to difficulties obtaining adequate cellular material. This is partly explained by an often desmoplastic growth pattern in CCA with depositions of fibrotic tissue around the tumor (191). Second, inter-observer variability for cytological categorization is high (Kappa coefficient, 0.59-0.66) (193, 194). Third, PSC is associated with conditions that affect the biliary epithelium and may mimic cellular atypia; biliary inflammation, bacterial cholangitis and prior instrumentation (195). The diagnostic performance of biliary brush cytology in PSC has been evaluated in a meta-analysis of 11 studies, including 747 patients (192). The pooled diagnostic values for detecting CCA were: sensitivity 43%, specificity 97%, positive predictive value (PPV) 78.2% and negative predictive value (NPV) 87.2%. Furthermore, the correlation between cellular atypia (w/o suspicion of malignancy) and presence or later development of CCA is not firmly established. In a study including 102 PSC patients with equivocal cytology results 30 (29%) was diagnosed with CCA within 2 years (196).

In order to enhance the diagnostic performance of biliary brush cytology assessment of chromosomal abnormalities has been introduced. Chromosomal abnormalities are manifest in up to 80% of biliary malignancies (197). Fluorescence *in-situ* hybridization (FISH) is a technique using DNA probes to detect loss or gain of chromosomes or chromosomal loci. In biliary malignancy a probe set to detect chromosomes 3, 7 and 17 and a locus-specific probe targeting 9p21 is the most widely used (198). Results are commonly categorized into trisomy, tetrasomy and polysomy. The diagnostic accuracy of FISH polysomy has been shown to be superior to other categories in a prospective study of 235 PSC patients with sensitivity, specificity, PPV and NPV of 46%, 88%, 55% and 84% respectively (199). In a meta-analysis including 6 studies and 690 PSC patients evaluated with FISH for polysomy, pooled results for sensitivity and specificity was 51% and 93% respectively (200). Two studies have also shown a strong association between FISH polysomy in serial or multifocal samplings and

development of CCA during long-term follow-up (201, 202). Furthermore, the use of FISH is suggested to be of higher value in the case of equivocal cytology results (196). In summary, detection of chromosomal abnormalities using FISH can increase sensitivity for CCA and identify PSC patients at greater risk for developing CCA but lacks specificity and its role in clinical use is not clear.

Peroral cholangioscopy is a method for visualizing bile ducts during ERCP and allows for visual characterization and targeted biopsies of suspected lesions (203). Conventional peroral cholangioscopy has disadvantages mainly related to that it requires two endoscopists for maneuvering. More recently, single-operator cholangioscopy (SOC) has been introduced for treatment of bile duct stones and assessment of strictures (204-206). Although this technique has demonstrated a high success rate for visualizing strictures and targeted sampling the utility of SOC in PSC has only been evaluated in a few patients (206).

2 AIMS

The general aim of this thesis was to explore pathogenic and diagnostic aspects of biliary strictures in primary sclerosing cholangitis with special focus on inflammation and malignancy.

Our specific aims were:

1. To evaluate the risk of adverse events in PSC patients undergoing ERCP (Paper I)
2. To evaluate PSC as a risk factor for procedure-related adverse events and specifically post-ERCP pancreatitis (Paper I)
3. To evaluate the feasibility, clinical utility and diagnostic accuracy of single-operator cholangioscopy with targeted sampling of biliary strictures in PSC (Paper II)
4. To evaluate the diagnostic accuracy of an algorithm for biliary brush cytology with sequential use of FISH in equivocal cases in patients with PSC (Paper III)
5. To describe the phenotype and function of MAIT cells in peripheral blood of PSC (Paper IV)
6. To establish a method for assessing MAIT cells in the biliary epithelium of PSC patients and control patients (Paper IV)

3 MATERIALS AND METHODS

The four studies included in this thesis are based on four different study cohorts. These cohorts have been generated using different methods, yet the study populations are partly overlapping. An overview of the study cohorts and generated papers is presented in Figure 4.

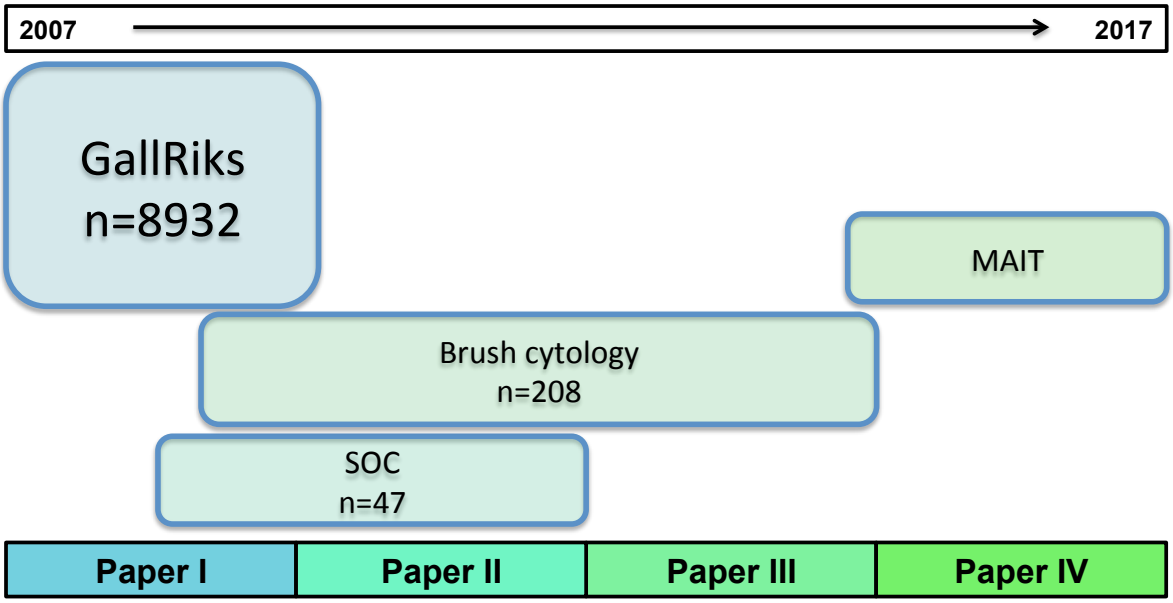


Figure 4 Cohorts and generated papers in this thesis

3.1 ETHICAL CONSIDERATIONS

All studies have ethical approval from the regional ethics committee of Stockholm County; dnr 2010/2105-31/2 (Paper I); dnr 2008/1588-32 (Paper II); dnr 2015/1963-31 (Paper III); dnr 2013/2285-31/3 and 2013/2084-31/2 (Paper IV). In studies that included prospective inclusion of patients (Paper II and IV), written consent was obtained from all participants.

3.2 PAPER I

3.2.1 Study population

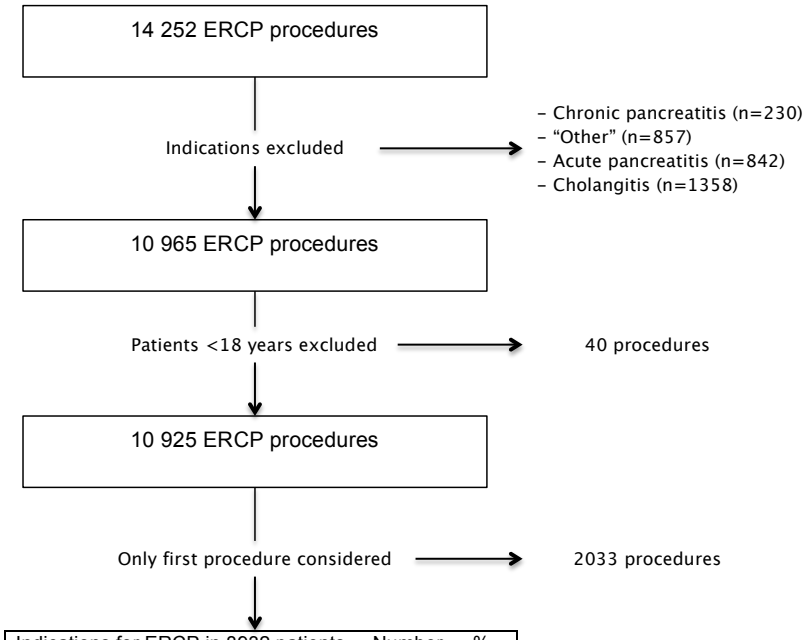
The study population was identified using the Swedish Registry for Gallstone Surgery and ERCP (GallRiks), a web-based nationwide quality registry. Details concerning registration and validation of data have previously been described (207). Between 1st of January 2007 and 31st of December 2009 we identified 14 252 procedures in 11 316 patients at 51 ERCP centers. Only the first procedure for each patient during the study period was considered to avoid selection bias. We excluded patients younger than 18 years and used registered indications for ERCP to further restrict the study cohort to patients who underwent ERCP with the intention to diagnose and treat biliary disease. Moreover, we excluded patients with the indications acute pancreatitis and cholangitis as these conditions might overlap with evaluated adverse events. The final study cohort included 8932 patients of whom 141 had PSC. Patient selection and indications for ERCP are described in Figure 5. When risk for the

adverse event cholangitis was calculated we excluded patients with ongoing treatment with antibiotics to avoid selection bias. For this analysis the cohort consisted of 7825 patients.

3.2.2 Definitions

Patients with PSC were defined according to the registered indication for ERCP; "PSC or suspicion of PSC". We further validated that this indication represented an actual diagnosis of PSC by retrieving medical records for all patients who underwent ERCP at Karolinska University Hospital in 2009 for cross-referencing. In 30 of 34 (88%) cases the diagnosis of PSC could be confirmed, which we considered acceptable.

Adverse events in GallRiks are categorized as intra-procedural or post-procedural. The endoscopist registers intra-procedural events in conjunction with the procedure. Appointed coordinators register post-procedural adverse events during a 30-day follow-up. Intra-procedural adverse events are bleeding and extravasation of contrast. An intra-procedural bleeding is defined as requiring intervention such as operation or transfusion. If the bleeding is controlled during the procedure, through for example diathermia, it is not registered. Extravasation of contrast is defined as contrast leak during the procedure. Post-procedural events include bleeding, cholangitis, pancreatitis and perforation. Bleeding is defined in patients with confirmed evidence of bleeding that requires transfusion and/or endoscopic or other re-interventions. Cholangitis is defined as a clinical diagnosis requiring hospitalization for intravenous antibiotics. Consensus criteria are used to define PEP (160). Perforation is registered in patients with bowel or bile duct perforations. Adverse events registered in GallRiks are not graded by severity.



Indications for ERCP in 8932 patients	Number	%
Common bile duct stone	3365	37.7
Malignancy	1384	15.5
Obstructive jaundice	3651	40.9
Stent dysfunction	190	2.1
Primary sclerosing cholangitis	141	1.6
Suspected bile leakage	116	1.3
Secondary profylaxis after previous gallstone pancreatits	85	1.0

Figure 5 Flowchart illustrating the selection of the patient cohort from GallRiks 2007–2009. Printed with permission.

3.2.3 Statistics

Differences between groups were calculated using unpaired 2-tailed student t-test, χ^2 test and Fischer exact test as appropriate. Backward stepwise logistic regression was used to fit a model for four categories of adverse events: PEP, cholangitis, extravasation of contrast and adverse events overall. The limited number of endpoints for the adverse events bleeding and perforation precluded the use of multivariate analysis in these categories. To select variables entered into the model, a list of explanatory values was compiled based on risk factors from previously published prospective studies (162-165, 208). To further select variables of importance we performed an initial univariate analysis for each of the categories of adverse events. The discriminating ability of the final predictive models selected by backward stepwise logistic regression was assessed using receiver-operating curves (ROCs). An area under the curve (AUROC) greater than or equal to 0.7 was considered as acceptable discrimination ability. Models were also assessed using goodness-of fit (Hosmer and Lemeshow test). Risk estimates are reported as Odds Ratios (ORs) and adjusted ORs (aORs).

The variables age and time were analyzed as a categorical variables. Based on previous studies comparing risk between precut and conventional sphincterotomy these variables were grouped together to increase power (166, 209). P-values less than 0.05 were considered significant. Analyses were carried out using SAS© System 9.1 (SAS Institute Inc., Cary, NC, USA) and STATISTICA 10.0 (Stat-soft®, Inc. Tulsa, OK, USA).

3.3 PAPER II

3.3.1 Study population

Paper II is a prospective single-center evaluation of the feasibility, safety and diagnostic accuracy of SOC with targeted sampling. The study was registered with ClinicalTrials.gov (NCT01556555) and approved by regional ethical committee in Stockholm County (2008/1588-32). Informed consent was signed by all participating patients.

Between September 2008 and May 2011 we prospectively enrolled patients with a diagnosis or suspicion of PSC referred to Karolinska University Hospital for ERCP. Clinical indications for ERCP in PSC patients at Karolinska University Hospital during the study period were (1) treatment of symptomatic bile duct strictures and (2) bile duct strictures with suspicion of malignancy, in adherence with the 2009 EASL guidelines (49). Exclusion criteria included; age <18 years, previous LTx and inability to provide informed consent. A total of 48 patients were included in the study and investigated with SOC (Spyglass direct visualization system, Boston Scientific Corp). One patient was diagnosed with secondary sclerosing cholangitis due to gall stone disease and excluded from the final analysis. A diagnosis of PSC was confirmed in the remaining 47 patients with cholangiographic changes demonstrated by MRCP and/or ERCP and exclusion of secondary causes. Patient enrollment and inclusion are illustrated in Figure 6.

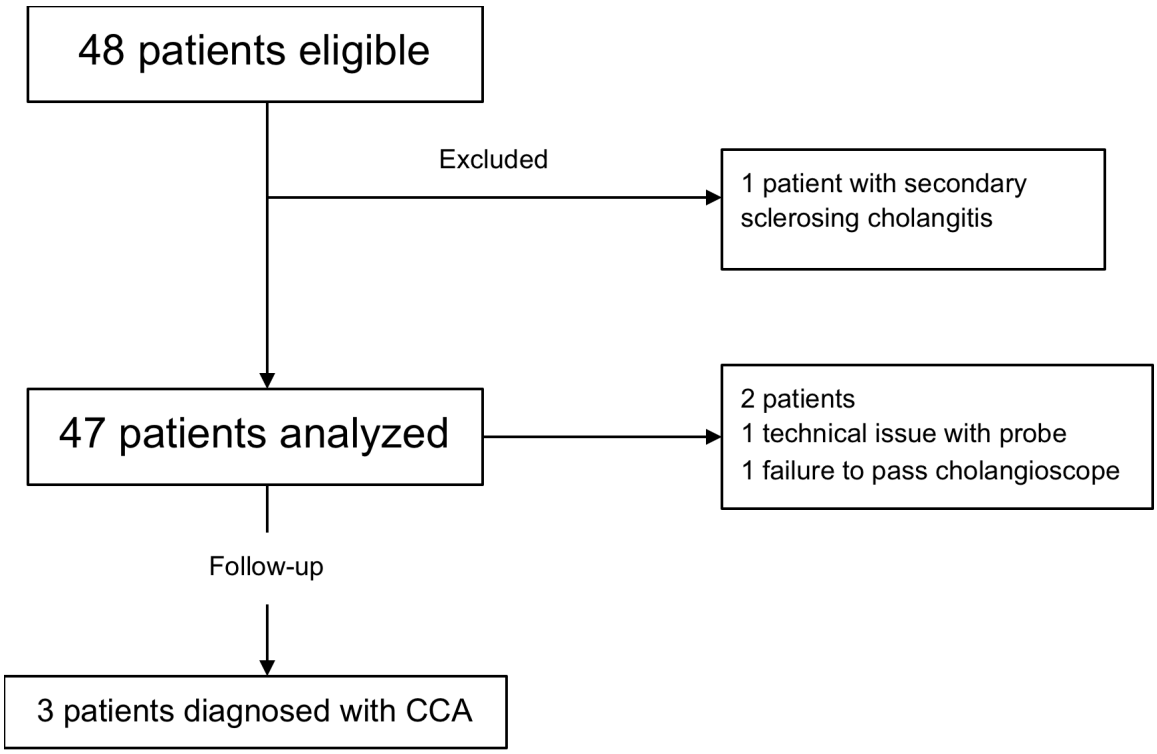


Figure 6 Flowchart illustrating patient inclusion and outcomes

3.3.2 ERCP procedure and SOC setting

All procedures were performed in general anesthesia. Antibiotic prophylaxis with four doses of piperacillin/tazobactam was administered at 8-hour intervals starting immediately before the ERCP in all patients. Patients were observed in hospital during at least an overnight stay. The Olympus duodenoscope (TJF160R or TJF160VR) together with the standard Spyglass direct visualization system (Boston Scientific Corp.) was used in all procedures. Sampling for biliary brush cytology and flow cytometry was routinely performed (210). The study protocol did not define criteria for which sampling method to use for evaluated strictures. Whenever visual findings were suspicious of dysplasia or malignancy guided biopsies by mini-forceps (Spybite; Boston Scientific Corp.) were taken at the discretion of the endoscopist. Focal lesions and strictures were categorized according to macroscopic features as normal, suspicious or malignant. Malignancy was defined as a focal lesion with mosaic-like asymmetric fibrotic areas, irregular red spots as a sign of neovascularization with bleeding-prone lesions with or without a papillar/polypoid. The study protocol did not include criteria for macroscopic inflammatory or postinflammatory changes of the bile duct mucosa.

3.3.3 Data collection

Clinical data registered included indication for ERCP, disease duration of PSC, IBD-phenotype and duration and serum liver function tests. During ERCP with SOC, the procedure was documented by multiple endoscopic and radiologic images. In conjunction with the procedure, data were collected in a structured questionnaire completed by the endoscopist. Information registered included Majoie Score (141), macroscopic features of the bile ducts at cholangioscopy, technical difficulties, quality of the procedure, and an overall

judgment of whether any abnormalities observed were benign or malignant. Patients were followed up until death, LTx, liver resection or until the study ended in March 2014. Clinical routine at our institution for PSC patients with suspicious strictures is to obtain liver function tests, and CA 19-9 at least every 6 months. Abdominal imaging by MRCP/MR or, if contraindicated, ultrasound is done annually.

3.3.4 Definitions and study outcomes

Criteria for suspicion of malignancy on MRCP included rapid progression of a stricture, markedly reduced contrast excretion in the segment proximal to the stricture and thickened bile duct wall on contrast-enhanced images. Conventional cytology specimens were evaluated by a cytopathologist and categorized as follows: inadequate cellularity, normal, reactive, atypical, suspicious for malignancy and positive for malignancy. Biopsy specimens where evaluated and categorized as: inadequate, normal, reactive, low-grade dysplasia, high-grade dysplasia and positive for adenocarcinoma.

To evaluate the feasibility, safety and diagnostic accuracy of SOC with targeted sampling we defined outcomes as procedure technical success, adverse events, sampling adequacy and diagnostic accuracy of sampling. A procedure technical success was defined when all the following criteria where met: (1) advancement of the cholangioscope to the targeted stricture, (2) adequate endoscopic visualization of the stricture and (3) sampling of the stricture by brush cytology or biopsy. Adverse events were defined and graded according to the 2010 consensus criteria of the American Society for Gastrointestinal Endoscopy (211). Diagnostic accuracy was defined as the accuracy of SOC-guided sampling (biopsy and/or brush cytology). For the purpose of this analysis all tissue samples categorized as reactive, atypical or suspicious of cancer were considered negative. The definite diagnosis was determined at the end of the follow-up period as: (1) Malignancy (CCA) defined as tissue pathology with evidence for adenocarcinoma. (2) Cancer-free, in patients with no evidence for cancer in tissue pathology from liver resection or LTx or by clinical course and imaging with no evidence of cancer. In addition to these outcomes we further defined the outcome additional technical value. This outcome was defined in patients where cholangioscopic guidance was used for advancement of the investigation to the targeted stricture.

3.3.5 Statistics

Descriptive statistics are presented as frequencies, median values and range. Analyses for diagnostic accuracy were done for procedures where technical success was achieved. The binomial exact method was used to obtain confidence interval for sensitivity, specificity, PPV and NPV. All analyses were carried out using STATA 13.1, StataCorp LP, College Station, TX, USA.

3.4 PAPER III

3.4.1 Study population

Paper III is a retrospective diagnostic study. We identified all patients with a diagnosis of large duct PSC who underwent ERCP with biliary brushings for cytology at Karolinska University Hospital between 1st January 2009 and 31st December 2015. Patients were identified through the pathology and cytology database, using topographic search terms for biliary brush cytology. This cohort was further linked to medical records to identify patients with PSC. All patients with a confirmed diagnosis of PSC and cholangiographic changes demonstrated by MRCP and/or ERCP (e.g. multifocal intra- and/or extrahepatic strictures, beading or narrowing of bile ducts) were included (49). Patients who had previously undergone LTx were excluded. The clinical indications for ERCP in PSC patients during that time at Karolinska University Hospital were (1) treatment of symptomatic bile duct strictures and (2) bile duct strictures with suspicion of malignancy in adherence with the 2009 EASL guidelines (49).

3.4.2 Data collection and definitions

Baseline clinical data (age, sex, duration of PSC, presence and duration of IBD) at the first (index) cytological brushing were retrieved from the electronic medical record system. Liver function tests up to 30 days prior to the index brushing and CA 19-9 +/- 30 days were recorded. Results of imaging features of MRI/MRCP within 4 months before the ERCP were categorized according to the original radiological reports as: (1) “Definite biliary malignancy”, when imaging revealed mass lesions consistent with CCA; (2) “Suspicion of malignancy”, when imaging demonstrated progressions of stricture(s), secondary signs of bile duct obstruction including reduced contrast excretion in the segment proximal of the stricture, and thickened bile duct wall on contrast-enhanced images and (3) “No suspicion of malignancy”, when none of the above criteria were fulfilled. The indication for ERCP was defined as: (1) “Malignancy”, when imaging results (CT or MRI/MRCP) revealed findings consistent with CCA; (2) Cholestatic symptoms (e.g. cholangitis, jaundice, pruritus) and (3) “Suspicion of malignancy”, when cholestatic symptoms or definite signs of malignancy on imaging were not present.

3.4.3 Brush Cytology and Fluorescence in Situ Hybridization

A stepwise procedure for the use of FISH in biliary brush cytology has been fully implemented since the 1st of January 2009 at Karolinska University Hospital. In short, brush cytology samples are categorized as benign (normal or reactive), equivocal (atypical or suspicious for malignancy) and malignant. Equivocal cases are analysed with FISH. Criteria for cytology and FISH are described below and the diagnostic algorithm is summarized in Figure 7.

Brush samples were collected in PreserveCyt® fixative (ThinPrep®, Hologic Inc.) and cells were transferred to glass slides using ThinPrep 5000. Following routine staining according to

Papanicolaou, the following alterations were registered: (1) Architectonic atypia; (2) Nuclear crowding/high n:c ratio; (3) Dissociation of atypical cells; (4) Hypercromasia and nuclear polymorphism; (5) Atypical nuclear membranes/envelopes; (6) Atypical nucleoli (shape and number); (7) Coarsely granular chromatin ("salt and pepper"). Based on the number of present criteria the samples were categorized as follows: inadequate cellularity, normal, reactive, atypical, suspicious for malignancy and positive for malignancy. When only 1-2 of these criteria were unequivocally present, the sample was categorized as non-malignant. When 3-5 of these criteria were present, the sample was also examined for aneuploidy using UroVysion® FISH (Abbot). With 6-7 criteria present the sample was categorized as positive for malignancy.

To analyze ploidy a new ThinPrep specimen was prepared. The slide was fixed for 10 minutes according to Carnoy (3 parts methanol and 1 part acetic acid), pretreated in 2xSSC for 2 minutes at 73°C, and taken for pepsin digestion (pepsin 10 mg/mL in 0.01M HCl) at 37°C for 10 minutes. Following washes in PBS the sample was refixed in 10% acid free formaldehyde, also containing 50mM MgCl2. After washing and dehydration through graded ethanols, the sample was air-dried. The UroVysion® FISH probes (Abbot) were added, denaturing the sample at 73°C for 2 minutes followed by hybridization at 39°C for 20 hours (overnight). Stringency wash was performed using 0.4xSSC with 0.4% IgepalCA-630 at 73°C for 2 minutes. After subsequent washings, first in 2xSSC containing 0.1% IgepalCA-630 followed by distilled water, the sample was left to dry in the dark. Nuclei were contrast stained by DAPI. Slides were mounted and analyzed in the UV microscope.

Interpretation followed the manufacturer´s recommendation, evaluating a minimum of 25 morphologically abnormal nuclei. Samples with less than 25 cells available for evaluation were recorded as insufficient. Malignant criteria were met when at least four nuclei showed increased number of fluorescent signals (exceeding three signals for at least two of the studied chromosomes 3, 7 or 17); or when at least 12 nuclei lacked both fluorescent signals for the p16INK4A gene located at 9p21 locus. Care was taken not to over interpret double chromosome settings (tetraploidy) or increased number of signals due to proliferating cells.

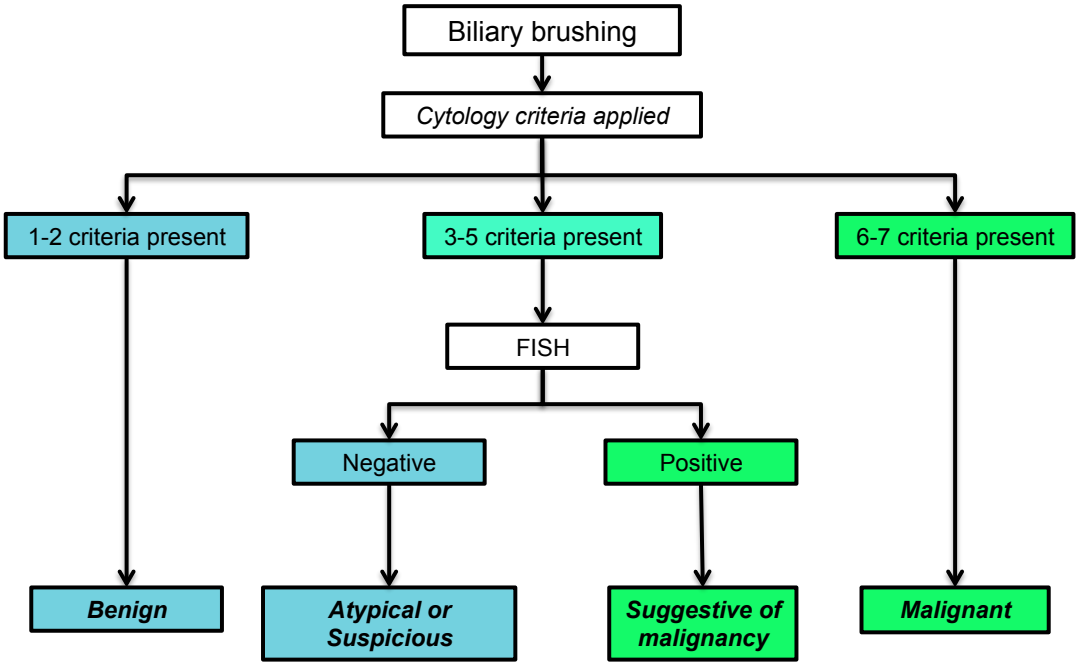


Figure 7 Flowchart illustrating the diagnostic algorithm for biliary brush cytology and sequential use of fluorescence in-situ hybridization for detection of cholangiocarcinoma

3.4.4 Reference standard and follow-up

The definite diagnosis of the evaluated strictures was determined after 12 months of follow-up. Data were collected from the electronic medical record system or, in cases were follow-up was done by other caregivers, by retrieving information in a standardized form. CCA was defined by the presence of an adenocarcinoma, including carcinoma in-situ, in biopsy specimens, explanted livers or liver resections. Presence of low- or high-grade dysplasia in surgical specimens was noted. In cases were a histopathological diagnosis was missing, CCA was defined as a mass lesion with typical features seen on cross-sectional imaging. Patients were classified as benign if a sufficient follow-up after 12 months showed no signs of malignancy. Clinical routine for PSC patients with significant biliary strictures at our institution is to obtain liver function tests, and CA 19-9 at least every 6 months. Abdominal imaging by MR/MRCP or, if contraindicated, by ultrasound is done annually. Patients without complete follow-up after 12 months were excluded from analyses.

The sensitivity, specificity, NPV and PPV was assessed using the result of the index biliary brushing during the study period. If simultaneous multiple brushings from different locations were performed the most severe result was registered for analysis of diagnostic accuracy. A positive index brush cytology test was defined as cytology positive for malignancy or with a positive FISH. A negative brush cytology index test was defined as cytology categorized as benign or atypical/suspicious with a negative FISH finding. All samples with inadequate cellularity were considered negative. Primary outcome measure was the diagnostic accuracy using CCA as positive endpoint. We also calculated diagnostic accuracy including HGD and LGD as positive endpoints.

3.4.5 Statistics

Descriptive statistics are presented as frequencies, median values and range. Confidence intervals for sensitivity, specificity, PPV and NPV were obtained using the exact binomial interval. All analyses were carried out using STATA 13.1, StataCorp LP, College Station, TX, USA.

3.5 PAPER IV

3.5.1 Study population

The study cohort of Paper IV consisted of patients recruited at the outpatient clinic or at the Endoscopy Unit at the Center for Digestive Diseases, Karolinska University Hospital. In addition healthy volunteers were also included. The study was approved by the regional ethics committee, Stockholm County (Dnr 2013/2285-31/3 and 2013/2084-31/2), and written informed consent was obtained from all participants. PSC patients had a confirmed diagnosis of PSC with cholangiographic changes demonstrated by MRCP and/or ERCP (e.g. multifocal intra- and/or extrahepatic strictures, beading or narrowing of bile ducts) (49). All UC patients had diagnosis based on clinical, endoscopic and histopathological findings consistent with diagnosis (212). Patients with PBC were likewise diagnosed upon typical clinical, biochemical and, in some cases, histopathological findings (49). Blood samples for analysis of peripheral blood mononuclear cells (PBMCs) were collected in healthy donors (n=12), UC patients (n=7), PBC patients (n=8) and PSC patients with and without IBD (n=2(). A second group of patients were sampled for analysis of PBMCs and biliary brush samples; PSC patients (n=8) and controls (n=12) (patients undergoing ERCP with biliary brush cytology for other indications than PSC).

3.5.2 Study material – collection and procession of samples

Peripheral blood mononuclear cells (PBMCs) were isolated by standard density-gradient separation and cryopreserved for deferred analysis.

Biliary brush samples were collected during ERCP. All samples were directly transferred into complete RPMI medium (RPMI 1640 medium (Thermo Fisher Scientific) containing 10% FCS (Thermo Fisher Scientific) and 1 mM L-glutamin (Invitrogen)) followed by an enzymatic digestion using collagenase II (0.25mg/mL, Sigma-Aldrich, St. Louis, Mo) and DNase (0.2 mg/mL; Roche, Mannheim, Germany) in RPMI 1640 medium without FCS for 15min at 37°C. After the digestion, complete RPMI medium was added and the cytobrushes were flushed with PBS. To increase cell yield, all medium/cell containing tubes were pooled, pelleted, and subsequently used for flow cytometry.

3.5.3 Flow cytometry

Cryopreserved PBMCs were thawed and stained with fluorescently labeled antibodies. Surface and intracellular staining was performed as previously described (213). Samples were acquired on a LSRFortessa flow cytometer (BD Biosciences) equipped with five lasers. Data

were analyzed using the FlowJo software. For high dimensional analysis of flow cytometry data, Barnes-Hut stochastic neighbor embedding (SNE) analysis was performed using R (The R Foundation for Statistical Computing) with an in-house built script as previously described (214).

3.5.4 In vitro MAIT cell functional assays

Functional analysis of MAIT cells was performed as previously described (213). Briefly, PBMCs were stimulated for 24 h with fixed E. coli or with a combination of IL-12 (10 ng/mL, Peprotech) and IL-18 (100 ng/mL, Medical & Biological Laboratories). To assess degranulation, anti-CD107a mAb was added at the beginning of the assay. In the E. coli-mediated stimulation assays, anti-MR1 mAb (clone 26.5, Biolegend) or IgG2a isotype control (clone MOPC-173, Biolegend) was added at the beginning of the assay at the final concentration of 20 μg/mL to assess which responses were MR1-dependent. Golgi Stop (Monensin, BD Biosciences) and Golgi Plug (brefeldin A, BD Biosciences) were added for the last 6 h of the incubation.

3.5.5 Statistical analyses

Statistical analyses were performed using Prism software v.6 (GraphPad). The distribution of all datasets was assessed by D'Agostino and Person normality test. Paired two-sided t-tests were performed for normally distributed data sets. Two-sided Wilcoxon matched-pairs signed rank test was used for non-normally distributed data sets. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

4 RESULTS

4.1 PAPER I

The final study cohort consisted of 8,932 patients, including 141 PSC patients, registered in GallRiks from the 1st of January 2007 until the 31st of December 2009.

PSC patients were younger than non-PSC patients (45 years ±16 vs. 69 years ±16, P<0.001) and showed a male predominance (62% vs. 44%, P<0.001). More interventions were done in the PSC-group such as balloon dilatations (29% vs. 4%, P<0.001), brushings (51% vs. 8%, P<0.001) and biopsies (8% vs. 4%, P=0.05). The procedure was also longer (51 min vs. 35 min, P<0.001) and prophylactic antibiotics given to a larger extent (49% vs. 36%, P<0.001). Patient- and procedure related characteristics are summarized in table 1.

During the follow-up period of 30-days a total of 33 adverse events occurred in the PSC group compared with 706 in the non-PSC group. The rate of adverse events overall was higher in PSC patients (18.4% vs. 7.3%, P<0.001), as was the occurrence of PEP (7.8% vs. 3.2%, P<0.002), cholangitis (7.1% vs. 2.1%, P<0.001) and extravasation of contrast (5.7% vs. 0.7%, P<0.001). Adverse events in PSC and non-PSC patients are summarized in table 2.

Table 1 Comparison of procedure related characteristics in 8932 PSC and non-PSC patients undergoing ERCP 2007-2009

Characteristics	PSC (n=141)	Non-PSC (n=8791)	P value
Hospital level			
University*	84 (60%)	2036 (23%)	<0.001
High-volume**	60 (43%)	1604 (18%)	<0.001
Priority – elective	115 (82%)	2656 (30%)	<0.001
Duration of procedure, Minutes (mean, SD)	51±34	35±22	<0.001
Prophylactic antibiotics	69 (49%)	3153 (36%)	0.002
Previous sphincterotomy	26 (18%)	709 (8%)	<0.001
Sphincterotomy	50 (35%)	5554 (63%)	<0.001
Precut sphincterotomy	15 (11%)	756 (9%)	0.854
Failed cannulation of bile duct	10 (7%)	854 (10%)	0.277
Cannulation of pancreatic duct	44 (31%)	2472 (28%)	0.419
Significant bile duct stricture	88 (62%)	2836 (32%)	<0.001
Balloon dilation of biliary stricture	41 (29%)	313 (4%)	<0.001
Pancreatic stent placement	2 (1.4%)	63 (0.6%)	0.417
Biliary stent placement	38 (27%)	3226 (37%)	0.017
Stent removal/replacement	9 (6%)	508 (6%)	0.760
Biopsy	11 (8%)	382 (4%)	0.05
Brush cytology	72 (51%)	714 (8%)	<0.001
Stone extraction	8 (6%)	2862 (33%)	<0.001
Cholangioscopy	20 (14%)	17 (0.2%)	<0.001

Table 2 Adverse events observed during prospective follow-up in PSC and non-PSC patients

Adverse events	PSC (n=141)	Non-PSC (n=8791)	P value
Any adverse event	26 (18.4%)	640 (7.3%)	<0.001
Post ERCP pancreatitis	11 (7.8%)	283 (3.2%)	0.002
Cholangitis	10 (7.1%)	188 (2.1%)	<0.001
Bleeding	1 (0.7%)	88 (1.0%)	0.729
Perforation	1 (0.7%)	32 (0.4%)	0.503
Extravasation of contrast	8 (5.7%)	60 (0.7%)	<0.001
Per-operative bleeding	2 (1.4%)	55 (0.6%)	0.241
Total adverse events	33 (23.6%)	706 (8.0%)	<0.001

4.1.1 Pancreatitis

PSC was a risk factor for PEP both by univariate analysis and in the final predictive model, OR 2.54 (95% CI 1.36 – 4.76) and aOR 2.02 (95% CI 1.04-3.92). Other identified independent risk factors were cannulation of the pancreatic duct, aOR 2.60 (95% CI 2.04-3.32) and female sex, aOR 1.32 (95% CI 1.04-1.68). A longer duration of the procedure was also associated with an increased risk of PEP. Factors associated with a decreased risk of PEP were presence of a biliary stent, aOR 0.38 (95% CI, 0.15-0.95), a significant bile duct stricture, aOR 0.63 (95% CI, 0.48-0.83) and high age. Table 3 and 4 shows crude ORs for all risk factors evaluated and aORs in the final predictive model. The prognostic model was well calibrated (Hosmer-Lemeshow p=0.32) and the AUROC-value was 0.70. Variables evaluated in the prognostic model were age, sex, procedure time, ASA classification, PSC, cannulation

of pancreatic duct, sphincterotomy classification, previous stent, biliary stent placement, pancreatic stent placement, stent extraction, cholangioscopy and significant bile duct stricture.

Notably, in a subgroup analysis of the PSC patients, the PEP-rate of patients with naïve papillas was 11/115 (9.6%) and none of the PSC patients who had a previous sphincterotomy (n=26) developed PEP. This group was however to small to make any reliable statistical inference on risk factors.

4.1.2 Cholangitis

PSC was associated with an increased risk of cholangitis by univariate analysis, OR 3.81 (95% CI 1.96 – 7.40). This association remained in the predictive model together with two more patient- and procedure-related variables; PSC, aOR 2.88 (95% CI, 1.47-5.65); a significant bile duct stricture, aOR 2.12 (95%, CI 1.55-2.89) and a longer duration of the procedure. Subgroup analysis did not show any association between prophylactic antibiotics and cholangitis in PSC patients (data not shown). Table 3 and 4 shows crude ORs for all risk factors evaluated and aORs in the final predictive model. The prognostic model was well calibrated (Hosmer-Lemeshow, p=0.74) and the AUROC-value was 0.66. Variables evaluated in the model were sex, procedure time, PSC, prophylactic antibiotics, sphincterotomy classification, dilation of biliary stricture, biliary stone extraction, previous stent, biliary stent placement, pancreatic stent placement, number of stents, stent extraction, biliary brush cytology, cholangioscopy and significant bile duct stricture.

4.1.3 Extravasation of contrast

The risk of extravasation of contrast was associated with variables signaling procedural technical problems. In the predictive model, increased risk was found for; PSC, aOR 5.84 (95% CI, 2.24-15.23), when the cannulation of the bile duct failed, aOR 6.34 (95% CI, 3.74-10.71) and if cannulation of the pancreatic duct was done, aOR 2.59 (95% CI, 1.56-4.29). Longer duration of the procedure and cholangioscopy, aOR 5.25 (95% CI, 1.31-20.92) were other variables associated with increased risk (table 3 and 4). The prognostic model was well calibrated (Hosmer-Lemeshow p=0.60) and the AUROC-value was 0.84. Variables included in the predictive model were procedure time, PSC, cannulation of biliary duct, cannulation of pancreatic duct, sphincterotomy classification, dilation of biliary stenosis, number of stents, biliary stone extraction, previous stent and biliary brush cytology.

4.1.4 Adverse events overall

The unadjusted risk for an adverse event was increased in PSC patients, OR 2.88 (95% CI 1.87 – 4.44). Four different variables were associated with increased risk for adverse events overall in the predictive model; PSC, aOR 2.11 (95% CI, 1.32-3.37), cannulation of the pancreatic duct, aOR 1.74 (95% CI, 1.46-2.05) and for dilation of a biliary stenosis, aOR 1.55 (95% CI, 1.07-2.23). The risk also increased with the time of the procedure. Factors associated with decreased risk were; biliary stone extraction aOR 0.81 (95% CI, 0.66-0.99),

the presence of a significant bile duct stricture, aOR 0.73 (95% CI, 0.61-0.91) and higher age (table 3 and 4). The prognostic model was well calibrated (Hosmer-Lemeshow p=0.57) and the AUROC-value was 0.65. Variables evaluated in the prognostic model were age, sex, procedure time, ASA classification, prophylactic antibiotics, PSC, cannulation of pancreatic duct, cannulation of bile duct, sphincterotomy classification, dilation of biliary stenosis, biliary stone extraction, previous stent, biliary stent placement, pancreatic stent placement, stent extraction, biliary brush cytology, cholangioscopy, number of stents and significant bile duct stricture.

Table 3 Unadjusted risk estimates for patient- and procedure related risk factors for adverse events overall, pancreatitis, cholangitis and extravasation of contrast. Values are odds ratios (ORs).

	Adverse events overall		**Pancreatitis**		**Cholangitis**		**Extravasation of contrast**	
Variable	n	Univariate	n	Univariate	n	Univariate	n	Univariate
Age								
>80 years	147	0.63 (0.50-0.78)*	49	0.41 (0.29-0.58)*	46	1.05 (0.68-1.60)	15	0.61 (0.31-1.19)
71-80 years	179	0.85 (0.69-1.05)	79	0.73 (0.55-0.99)*	48	1.15 (0.75-1.74)	13	0.58 (0.29-1.16)
61-70 years	135	0.76 (0.61-0.95)*	61	0.67 (0.49-0.93)*	35	0.98 (0.62-1.53)	18	0.96 (0.51-1.80)
18-60 years	205	Reference group	105	Reference group	43	Reference group	22	Reference group
Sex								
Women	377	1.06 (0.90-1.24)	180	1.29 (1.02-1.64)*	97	0.88 (0.65-1.19)	40	1.16 (0.71-1.88)
Man	289	Reference group	114	Reference group	81	Reference group	28	Reference group
ASA score								
3-5	322	0.91 (0.78-1.07)	124	0.71 (0.56-0.90)*	95	1.23 (0.91-1.67)	35	1.04 (0.64-1.67)
1-2	344	Reference group	170	Reference group	77	Reference group	33	Reference group
Priority								
Urgent	444	0.89 (0.75-1.05)	179	0.69 (0.54-0.88)*	120	1.22 (0.88-1.69)	45	0.87 (0.52-1.44)
Elective	222	Reference group	115	Reference group	52	Reference group	23	Reference group
PSC	26	2.88 (1.87-4.44)*	11	2.54 (1.36-4.76)*	10	3.81 (1.96-7.40)*	8	8.65 (4.05-18.44)*
Profylactic antibiotics	252	1.08 (0.91-1.28)	111	1.04 (0.81-1.33)	73	1.05 (0.78-1.43)	32	1.64 (0.99-2.73)
Procedure time								
>45 min	235	2.86 (2.27-3.61)*	97	2.40 (1.71-3.37)*	53	2.56 (1.61-4.06)*	35	8.83 (3.45-22.58)*
31-45 min	170	2.04 (1.60-2.61)*	70	1.74 (1.21-2.50)*	50	2.41 (1.51-3.84)*	20	5.12 (1.92-13.67)*
21-30 min	148	1.58 (1.23-2.03)*	74	1.66 (1.16-2.38)*	41	1.77 (1.09-2.87)*	8	1.85 (0.60-5.66)
≤20 min	113	Reference group	53	Reference group	28	Reference group	5	Reference group
Significant bile duct stricture	220	1.01 (0.86-1.20)	77	0.72 (0.55-0.94)*	95	2.70 (1.82-4.01)*	26	1.24 (0.76-2.03)
Sphincterotomy								
Sphincterotomy and/or precut	515	1.06 (0.87-1.31)	219	0.79 (0.60-1.04)	128	1.33 (0.85-2.01)	52	0.84 (0.46-1.52)
Previous Sphincterotomy	31	0.55 (0.37-0.83)*	7	0.22 (0.10-0.48)*	19	1.72 (0.94-3.15)	2	0.30 (0.07-1.33)
No	120	Reference group	68	Reference group	25	Reference group	14	Reference group
Cannulation of bile duct								
Failed cannulation								
Superficial cannulation	98	1.69 (1.34-2.12)*	35	0.79 (0.55-1.14)	11	0.64 (0.34-1.18)	29	7.66 (4.66-12.59)*
Not attempted	14	1.42 (0.81-2.48)	2	2.38 (0.59-9.64)	6	2.17 (0.94-5.01)	4	6.30 (2.21-17.97)*
Deep cannulation	8	0.64 (0.31-1.32	6	0.92 (0.40-2.10)	1	0.29 (0.04-2.05)		NA
	546	Reference group	251	Reference group	154	Reference group	35	Reference group
Cannulation of pancreatic duct	292	2.12 (1.80-2.49)*	158	3.09 (2.44-3.91)*	61	1.41 (1.02-1.93)*	41	3.91 (2.40-6.38)*
Previous stent	31	0.62 (0.43-0.89)*	5	0.22 (0.09-0.53)*	17	1.49 (0.90-2.48)	1	0.19 (0.03-1.37)
Stent extraction	27	0.67 (0.45-0.99)*	5	0.27 (0.11-0.67)*	16	1.67 (0.99-2.82)*	0	
Biliary stone extraction	186	0.81 (0.67-0.96)	82	0.81 (0.63-1.05)	35	2.23 (1.64-3.02)*	6	0.20 (0.09-0.46)*
Dilation of biliary stenosis	45	1.87 (1.35-2.58)*	14	1.22 (0.71-2.11)	20	3.34 (2.07-5.40)*	7	2.76 (1.25-6.08)*
Biliary stent placement	239	0.97 (0.82-1.14)	96	0.84 (0.65-1.07)	96	2.23 (1.64-3.02)*	20	0.70 (0.21-1.18)
Pancreatic stent placement	11	2.55 (0.86-1.19)	8	4.21 (1.99-8.91)*	0		1	2.87 (0.39-21.11)
Number of stents								
≥2	17	0.84 (0.51-1.39)	6	0.65 (0.29-1.48)	7	2.02 (0.92-4.43)	1	0.43 (0.06-3.17)
1	233	1.03 (0.87-1.21)	98	0.94 (0.74-1.21)	89	2.16 (1.59-2.95)*	20	0.76 (0.45-1.29)
0	416	Reference group	190	Reference group	76	Reference group	47	Reference group
Brush cytology	70	1.24 (0.96-1.60)	30	1.18 (0.81-1.74)	25	1.66 (1.08-2.56)*	10	1.77 (0.90-3.48)
Biospy	29	0.98 (0.67-1.45)	15	1.17 (0.69-2.00)	6	0.73 (0.32-1.66)	3	1.01 (0.32-3.24)
Cholangioscopy	10	4.65 (2.24-9.65)	4	3.60 (1.27-10.22)	2	2.64 (0.63-11.06)	4	16.92 (5.81-49.22)*

* $p < 0.05$

Table 4 Adjusted risk estimates for patient- and procedure related risk factors from predictive models for adverse events overall, pancreatitis, cholangitis and extravasation of contrast. Values are adjusted odds ratios (aORs).

	Adverse events overall		Pancreatitis		Cholangitis		Extravasation of contrast	
Variable	n	Multivariate	n	Multivariate	n	Multivariate	n	Multivariate
Age								
>80 years	147	0.65 (0.52-0.82)*	49	0.42 (0.30-0.60)*	46		15	
71-80 years	179	0.86 (0.69-1.07)	79	0.75 (0.55-1.03)	48		13	
61-70 years	135	0.76 (0.60-0.96)*	61	0.70 (0.50-0.97)*	35		18	
18-60 years	205	Reference group	105	Reference group	43		22	
Sex								
Women	377		180	1.32 (1.04-1.68)*	97		40	
Man	289		114	Reference group	81		28	
PSC	26	2.11 (1.32-3.37)*	11	2.02 (1.04-3.92)*	10	2.88 (1.47-5.65)*	8	5.84 (2.24-15.23)*
Procedure time								
>45 min	235	2.43 (1.90-3.01)*	97	2.02 (1.41-2.88)*	53	2.09 (1.31-3.34)*	35	3.95 (1.49-10.45)*
31-45 min	170	1.86 (1.45-2.39)*	70	1.55 (1.07-2.25)*	50	2.06 (1.29-3.31)*	20	2.99 (1.10-8.11)*
21-30 min	148	1.54 (1.20-1.99)*	74	1.63 (1.13-2.34)*	41	1.60 (0.98-2.61)	8	1.49 (0.48-4.59)
≤20 min	113	Reference group	53	Reference group	28	Reference group	5	Reference group
Significant bile duct stricture	220	0.73 (0.61-0.91)*	77	0.63 (0.48-0.83)*	95	2.12 (1.55-2.89)*	26	
Sphincterotomy								
Sphincterotomy and/or precut	515	1.10 (0.88-1.37)	219	0.70 (0.53-0.93)*	128		52	
Previous Sphincterotomy	31	0.63 (0.42-0.96)*	7	0.32 (0.14-0.73)*	19		2	
No	120	Reference group	68	Reference group	25		14	
Cannulation of bile duct								
Failed cannulation								
Superficial cannulation	98		35		11		29	6.34 (3.74-10.71)*
Not attempted	14		2		6		4	4.61 (1.59-13.41)*
Deep cannulation	8		6		1			NA
	546		251		154		35	Reference group
Cannulation of pancreatic duct	292	1.74 (1.46-2.05)*	158	2.60 (2.04-3.32)*	61		41	2.59 (1.56-4.29)*
Previous stent	31		5	0.38 (0.15-0.95)*	17		1	
Biliary stone extraction	186	0.81 (0.66-0.99)*	82		35		6	
Dilation of biliary stenosis	45	1.55 (1.07-2.23)*	14		20		7	
Cholangioscopy	10		4		2		4	5.25 (1.31-20.92)*

NA –not applicable, * p< 0.05

4.2 PAPER II

The final study cohort consisted of 47 PSC patients with a median age of 40 (range 22-70), and male predominance (70%). The indications for ERCP were: jaundice in 28% (13/47); a suspected malignant stricture on MRCP in 62% (29/47); and need of stent exchange in 9% (5/47). Table 5 shows the clinical characteristics of patients included.

4.2.1 Technical evaluation – feasibility

The SOC procedure was considered technically successful in 96% (45/47) of cases. In the two unsuccessful investigations one failed due to technical issues with the SOC-probe and one due to difficulties to pass the scope into the biliary duct. Table 6 summarizes clinical information and technical aspects of the ERCP and SOC procedure in the 45 patients with a

technical success. In total, 64 strictures were evaluated with SOC: 23% (15/64) were present in the common bile duct, 20% (13/64) in the common hepatic duct, 11% (7/64) were hilar strictures, 34% (22/64) were strictures of the left or right hepatic duct and 11% (7/64) were segmental duct strictures. Table 7 summarizes the location, appearance and sampling of the 64 strictures evaluated. Strictures were inspected and passed in 50 cases and only inspected but not passed in 14. The locations of the investigated biliary strictures are shown in Figure 8.

Table 5 Clinical characteristics and indications for ERCP with SOC in 45 PSC patients

Age, median (range), years	40 (22-70)
Duration of PSC at time of SOC investigation, median (range), years	6,4 (0-35.9)
Male sex, n (%)	33 (70%)
Follow-up time, median (range), months	27 (2-64)
IBD	
Ulcerative colitis, n (%)	30 (64%)
Crohn's Disease, n (%)	6 (13%)
No IBD, n (%)	11 (23%)
Indication for ERCP/SOC	
Jaundice, n (%)	13 (28 %)
Stricture with suspicion of malignancy, n (%)	29 (62%)
Change or extraction of stent, n (%)	5 (9%)

Table 6 Summary of clinical information and technical aspects of ERCP procedures with SOC in 45 patients with PSC

	n (%)
Endoscopic sphincterotomy (ES)	
No ES	5 (11%)
ES performed	13 (29%)
ES previously performed	27 (60%)
Presence of bile duct stones	12 (27%)
Successful removal of the stones	11 (92%)
Majoie Score	
Intrahepatic	
Type 0	0
Type I	8 (18%)
Type II	34 (76%)
Type III	3 (7%)
Extrahepatic	
Type 0	2 (4%)
Type I	10 (22%)
Type II	25 (56%)
Type III	8 (18%)
Type IV	0

Table 7 Information on location, sampling and macroscopic appearance of 64 strictures in 45 PSC patients evaluated with SOC

	n (%)
Location of investigated strictures	
Common bile duct	15 (23%)
Common hepatic duct	13 (20%)
Hilar	7 (11%)
Left or right hepatic duct	22 (34%)
Segmental ducts	7 (11%)
Biliary brush samples	63 (98%)
Biopsy by mini-forceps	22 (34%)
Passed strictures	50 (78%)
Strictures inspected	
Before dilatation	50 (78%)
After dilatation	13 (20%)
After stone extraction	1 (2%)
Macroscopic appearance of strictures	
Malignant	1 (2%)
Suspicious	3 (5%)
Normal	60 (93%)

4.2.2 Adverse events

Adverse events occurred in a total of 15% percent (7/47) of patients. Mild PEP was diagnosed in 4 patients. Cholangitis was seen in 2 patients. One patient had mild cholangitis treated with per oral antibiotics. One patient had severe bacterial cholangitis and cholecystitis. A majority of all adverse events (71%, 5/7) occurred in the first 15 patients.

4.2.3 Sampling, end-points and diagnostic accuracy

Sampling results of biliary brush cytology and ductal biopsies are presented in table 8. Cellularity was sufficient in 95% (21/22) of the mini-forceps biopsies and in 98% (62/63) of the biliary brushings. The median follow-up time was twenty-eight months (range 2 – 64 months) during which three patients were diagnosed with CCA. One patient presented with a macroscopic appearance categorized as malignant on cholangioscopy and findings positive for malignancy on both cytology and biopsy. The second patient had an intrahepatic CCA diagnosed 17 months after inclusion. The third patient was diagnosed with a perihilar CCA at the time of LTx after 35 months of follow-up. Of the remaining 42 PSC patients, 19 underwent LTx with no signs of CCA in the explanted liver although HGD was detected in 4. Two patients with strong suspicion of cancer underwent hepatic resection. The resected specimens were negative for cancer in both cases. Median follow-up time in the 21 patients with final status determined by clinical course was 47 months (range 17-64).

The sensitivity of detection of CCA with cholangioscopy-guided sampling was 33% (95% CI 1-91%), the specificity 100% (95% CI 92-100%), PPV 100% (95% CI 3-100%), NPV 95% (95% CI 85-99%) and diagnostic accuracy 96% (95% CI 85-100%).

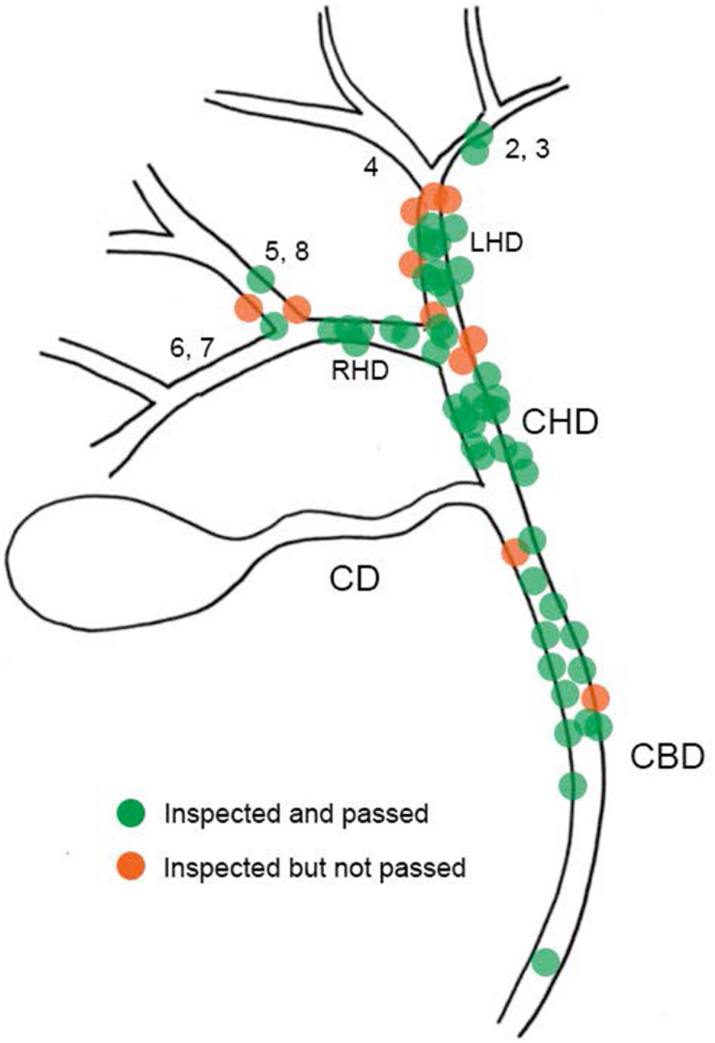


Figure 8 Location of 64 strictures in 45 patients with PSC, and whether the cholangioscope could pass the stricture. CBD, common bile duct; CHD, common hepatic duct; CD, cystic duct; LHD, left hepatic duct; RHD, right hepatic duct. Numbers indicate segmental bile ducts

4.2.4 Additional technical value

The criteria for the outcome technical advantage was met in four patients (9%) were the target lesion could not have been reached without the options offered by the cholangioscopic visualization. Notably, bile duct stones were found in 12 cases (27%); these could be evacuated and the strictures dilated. In 20% (13/64) of cases the stricture required dilatation before inspection.

4.2.5 Endoscopic appearance of bile ducts in PSC

A thin fibrotic circular narrowing of the bile duct, which was easily dilated, was a common finding. These lesions were observed either proximal or distal to a stricture, or sometimes both. In three patients strictures with an appearance that raised suspicion of malignancy were found. None of them had sampling results categorized as dysplastic (data not shown). Furthermore, all four patients with HGD detected in explanted livers were considered to have benign conditions at endoscopy.

Table 8 Sampling results of biliary brush cytology and ductal biopsies of evaluated strictures in 45 PSC patients

	Result					
	Inadequate	**Normal**	**Reactive**	**Atypical**	**Suspicious**	**Malignancy**
Brush cytology (n=63)	1 (2%)	45 (71%)	2 (3%)	7 (11%)	6 (10%)	2 (3%)
	Inadequate	**Normal**	**Reactive**	**LGD**	**HGD**	**Malignancy**
Biopsy (n=22)	1 (5%)	13 (59%)	3 (14%)	0 (0%)	4 (18%)	1 (5%)

4.3 PAPER III

During the study period, 2009-2015, a total of 214 PSC patients underwent ERCP with biliary brush cytology. Incomplete follow-up, other or unidentified malignancy in 6 patients left 208 individuals eligible for analysis. The study design, brush cytology results and endpoints are summarized in Figure 9. The median age of the patients was 40.8 years and 68% were men. The most common indication for ERCP with brushings was stricture(s) with suspicion of malignancy (77%), followed by cholestatic symptoms (22%). In two patients (1%) cross-sectional imaging showed definite signs of a biliary malignancy prior to the ERCP. Clinical characteristics and indications for ERCP are presented in Table 9.

4.3.1 Cytology results and endpoints

Most patients (68%) had benign cytological findings and one sample (0.5%) was reported malignant based on cytomorphology from the index brushing. In the remaining 62 patients with equivocal cytology, a positive FISH result was detected in 31%. Results of the index biliary brush cytologies are presented in Table 10. Insufficient material for conventional cytology was low (1%), although higher for FISH analysis, 15-17% was found to be insufficient when the definition of more than 25 morphologically abnormal nuclei available for FISH evaluation was applied.

CCA was confirmed in 15 patients (7%) based on histology and/or imaging during follow-up. A histopathological diagnosis was made from the following specimens: Biopsy from primary tumor or metastasis (n=3); surgical resection (n=5) and; explanted liver (n=6). Radiographic findings alone confirmed the cancer diagnosis in one patient. In total, 45 patients underwent LTx, liver resection or had a biopsy from suspected lesions during the 12 months' follow-up. Among the 34 transplanted patients, 3 (10%) were shown to have HGD, and 5 (17%) to have LGD in the explanted liver. Diagnostic endpoints after 12 months of follow-up are summarized in Table 11 and Figure 9.

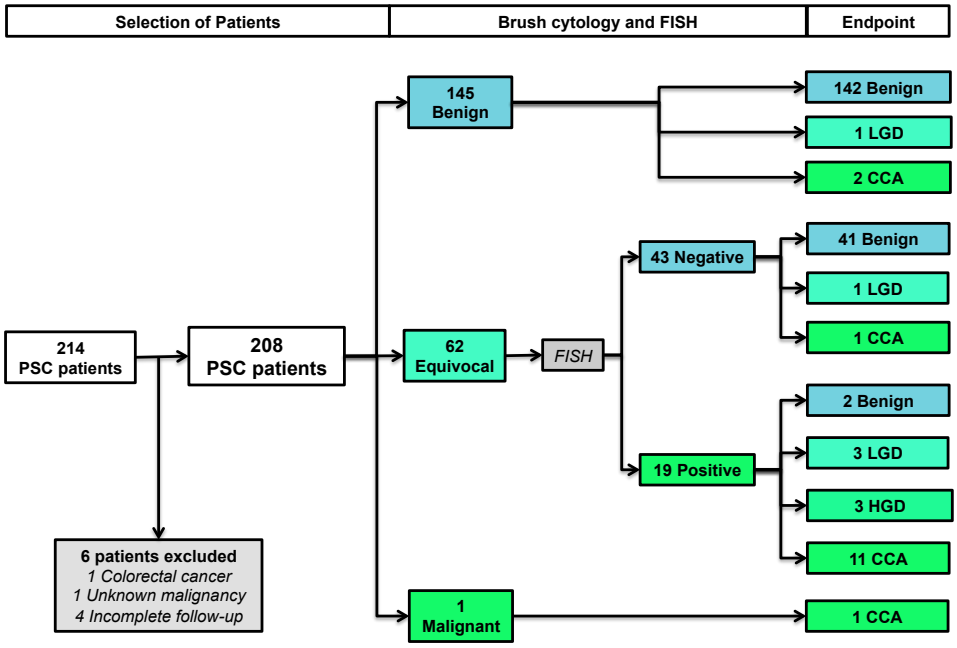


Figure 9 Summary of inclusion process, results from biliary brush cytology and FISH, and end-points after 12 months of follow-up

4.3.2 Diagnostic performance

The diagnostic performance for detection of CCA in all 208 cases by brush cytology, including FISH in equivocal cases, had a sensitivity, specificity, PPV, NPV and accuracy of 80% (95%CI 52-96%), 96% (95%CI 92-98%), 60% (95%CI 36-81%), 98% (95%CI 95-100%) and 95% (95% CI 91-97%), respectively.

To rule out biliary dysplasia and CCA in patients with PSC is well known to be inconceivable. Therefore, we tested the diagnostic performance in the subgroup of patients in whom a confirmed follow-up diagnosis were obtained by either LTx, liver resection, biopsy or cancer related death (n=46). For CCA and HGD the sensitivity, specificity, NPV decreased to 83% (95% CI 59-96%), 82% (95% CI 63-94%), and 89% (95% CI 70-98%) and the PPV increased to 75% (95% CI 51-91%). The diagnostic performance for detection of CCA, HGD and LGD by routine cytology and FISH is summarized in Table 12.

4.3.3 Diagnostic performance in patients with equivocal cytology

In clinical practice, a major problem is the interpretation of equivocal cytology in patients without conclusive imaging results. Current guidelines recommend FISH in equivocal cases (47). Therefore, we investigated the diagnostic accuracy in this subgroup of patients. Sensitivity, specificity, NPV, PPV and accuracy were calculated in patients with equivocal brush cytology results and no evident malignancy on imaging prior to sampling (n=61). A positive FISH result was detected in 19 (31%) of these patients and 11 (18%) were diagnosed

with CCA during follow-up. Sensitivity, specificity, PPV and NPV for CCA alone in this group was 100% (95%CI 72-100%), 84% (95%CI 71-93%), 58% (95%CI 34-80%) and 100% (95%CI 92-100%). Results for detecting CCA, HGD and LGD are presented in Table 12.

Table 9 Clinical characteristics and results of serum CA 19-9 and imaging in 208 PSC patients at inclusion

Age, years (median, range)	40.8 years (18.4-77.5)
Sex, n (%) Female Male	 66 (32%) 142 (68%)
IBD, n (%) No IBD UC Mb Crohn Indeterminate colitis Unknown	 33 (16%) 135 (65%) 33 (16%) 5 (2%) 2 (1%)
Duration of PSC, years (median, range)	5.6 (0-41)
Bilirubin (median, range) (n=197) (Normal range <26µmol/L)	14 mmol/L (3-410)
ALP (median, range) (n=183) (Normal range <1,9 µkat/L)	3.7 µkat/L (0.7-30.3)
CA 19-9 (median, range) (n=117) <20 kU/L n,(%) <120 kU/L n (%)	15 kU/L (0.3-18220) 50 (43%) 16 (14%)
Results of MRI/MRCP (n=121) Definite biliary malignancy**, n (%)** Suspicion of malignancy**, n (%)** No suspicion of malignancy**, n (%)**	 1 (1%) 71 (59%) 49 (40%)
Indications for ERCP Malignancy**, n (%)** Cholestatic symptoms**, n (%)** Stricture with suspicion of malignancy**, n (%)**	 2 (1%) 45 (22%) 161 (77%)

Table 10 Results of biliary brush cytology and FISH analysis in 208 PSC patients at the index ERCP

Conventional cytology	**n (%)**	**FISH**	**n (%)**
Inadequate cellularity	3 (1%)		-
Benign	142 (68%)		-
Atypical	33 (16%)	Insufficient material Negative Positive	5 (15%) 27 (82%) 1 (3%)
Suspicious for malignancy	29 (14%)	Insufficient material Negative Positive	5 (17%) 6 (21%) 18 (62%)
Malignancy	1 (0.5%)		-

Table 11 Endpoints and method of ascertainment after 12 months of follow up in 208 PSC patients undergoing biliary brush cytology

Final diagnosis **Method for diagnosis**	**Benign**	**CCA**	**HGD**	**LGD**	**Total**
Clinical follow up with no signs of malignancy	162	-	-	-	**162**
LTx	20	6	3	5	**34**
Surgical resection **Liver resection** **Whipple**	 3 -	 3 2	 - -	 - -	 **6** **2**
Biopsy	-	3	-	-	**3**
Cross-sectional imaging only	-	1	-	-	**1**
Total	**185**	**15**	**3**	**5**	**208**

Table 12 Diagnostic performance of biliary brush cytology with sequential FISH for detecting CCA and dysplasia in index brushing of 208 PSC patients and in 61 PSC patients with equivocal cytology and no definitive evidence for CCA on imaging.

Diagnostic performance in all patients (n=208)					
	Sensitivity % (95%CI)	**Specificity % (95%CI)**	**PPV % (95%CI)**	**NPV % (95%CI)**	**Accuracy (95% CI)**
CCA	80% (52-96%)	96% (92-98%)	60% (36-81%)	98% (95-100%)	95% (91-97%)
Analysis restricted to patients with confirmed diagnosis by LTx, surgical resection, biopsy or cancer related death (n=46)					
CCA	80% (52-96%)	74% (55-88%)	60% (36-81%)	89% (70-98%)	76% (61-87%)
CCA+HGD	83% (59-96%)	82% (63-94%)	75% (51-91%)	89% (70-98%)	82% (69-92%)
CCA+HGD+LGD	78% (56-93%)	91% (72-99%)	90% (68-99%)	81% (61-93%)	85% (71-94%)
Diagnostic performance of FISH in patients with equivocal cytology without definitive evidence of CCA on imaging (n=61)					
CCA	100% (72-100%)	84% (71-93%)	58% (34-78%)	100% (92-100%)	87% (76-94%)
CCA+HGD	100% (77-100%)	55% (23-83%)	74% (49-91%)	100% (54-100%)	80% (59-93%)
CCA+HGD+LGD	94% (73-100%)	71% (29-96%)	90% (67-99%)	83% (36-100%)	88% (69-97%)

4.4 PAPER IV

4.4.1 MAIT cells in peripheral blood of PSC patients

MAIT cells can be defined in humans as T cells expressing the TCR Vα7.2 segment in combination with expressing high levels of CD161 (Figure 10, and 11A) (215). We first analyzed the frequency of MAIT cells in patients with PSC compared to IBD and PBC as well as to healthy controls (see Table 13 for patient characteristics). The frequency of MAIT cells was reduced in the PSC patients (median: 0.75%, range: 0.13-5.2%) as compared to healthy controls (median: 2.23%, range: 0.57-4.40%) (Figure 11B). A similar reduction in MAIT cells was also noted for IBD and PBC patients (Figure 11B). The Vα7.2+CD161- T cell population was used as a reference population. When analyzing the levels of these cells, no difference was noted in the patient cohorts as compared to healthy controls (Figure 11B). The MAIT cell loss affected both the CD8+ and the CD8- MAIT cell subsets (Figure 11C). Taken together, these data indicate that the peripheral blood MAIT cell population was substantially reduced in PSC. However, since similar reductions were noted within disease control cohorts (IBD, intestinal inflammation; PBC, autoimmune liver inflammation), this

loss did not appear to be specific for PSC but rather a common feature of chronic inflammatory disorders affecting these organs.

Figure 10 Gating scheme to identify MAIT cells. To identify MAIT cells, first doublet events were excluded followed by identification of lymphocytes, removal of $CD14^{+}CD19^{+}$ and dead cells, gating for $CD3^{+}$ cells, subsequent gating for $CD161^{+}V\alpha7.2^{+}$ cells, and finally removal of $CD4^{+}$ cells

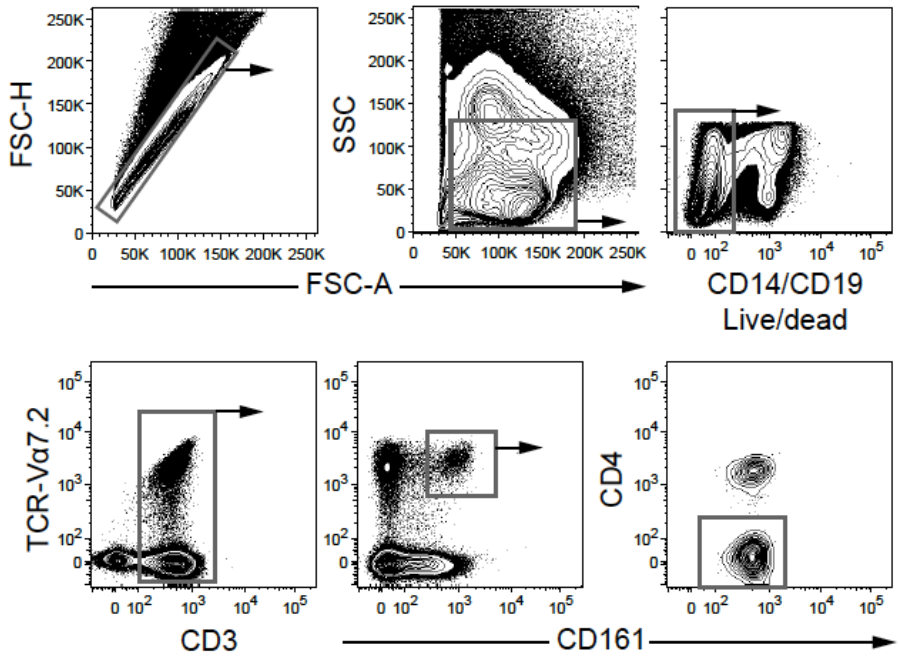
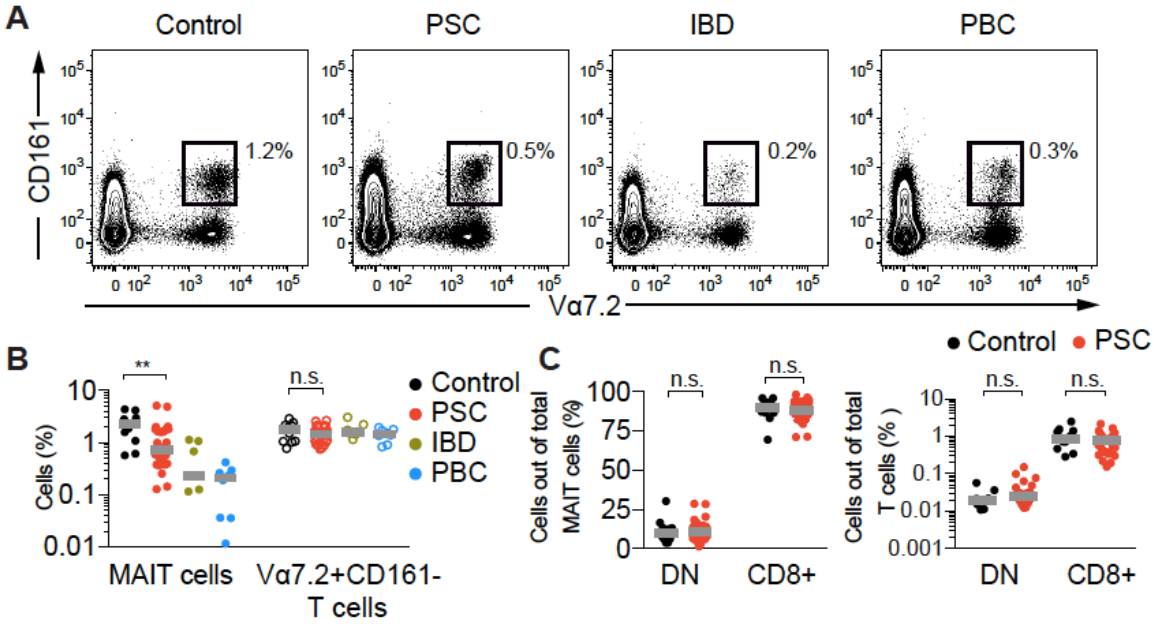


Table 13 Clinical characteristics of study subjects from which MAIT cells in peripheral blood were analyzed (PBMC-cohort)

	Healthy donors (n=12)	**PSC (n=28)**	**IBD (n=7)**	**PBC (n=8)**
Male sex, %	50% (6/12)	71% (20/28)	57% (4/7)	38% (3/8)
Age, years (median, range)	33 (27-41)	41 (26-73)	48 (28-72)	70 (51-79)
PSC duration, years (median, range)	NA	10 (2-28)	NA	NA
IBD, % **Ulcerative colitis** **Mb Crohn** **Indeterminate colitis**	NA	82% (23/28) 78% (18/23) 22% (5/23) 0% (0/23)	100% (7/7)	NA
Bilirubin, µmol/L (median, range)	NA	10.0 (5-31)	NA	7 (5-82)
ALP, µkat/L (median, range) **Proportion >ULN (%)**	NA	1.9 (0.6-10.0) 50% (14/28)	NA	1.9 (1.0-3.9) 38% (3/8)

ULN –upper limit of normal range

Figure 11. (A) Representative flow cytometry plots for identification of Vα7.2^{+}CD161^{+} MAIT cells in patients and controls. **(B)** Percentage of MAIT cells, and Vα7.2^{+}CD161^{-} T cells, out of total T cells in peripheral blood of healthy controls (*n*=12), patients with PSC (*n*=28), IBD (*n*=7), and PBC (*n*=8). **(C)** Frequency of CD8^{+} or double-negative MAIT cells out of total MAIT cells or total T cells in healthy controls and PSC patients.



4.4.2 High-dimensional SNE analysis of peripheral blood MAIT cells in PSC

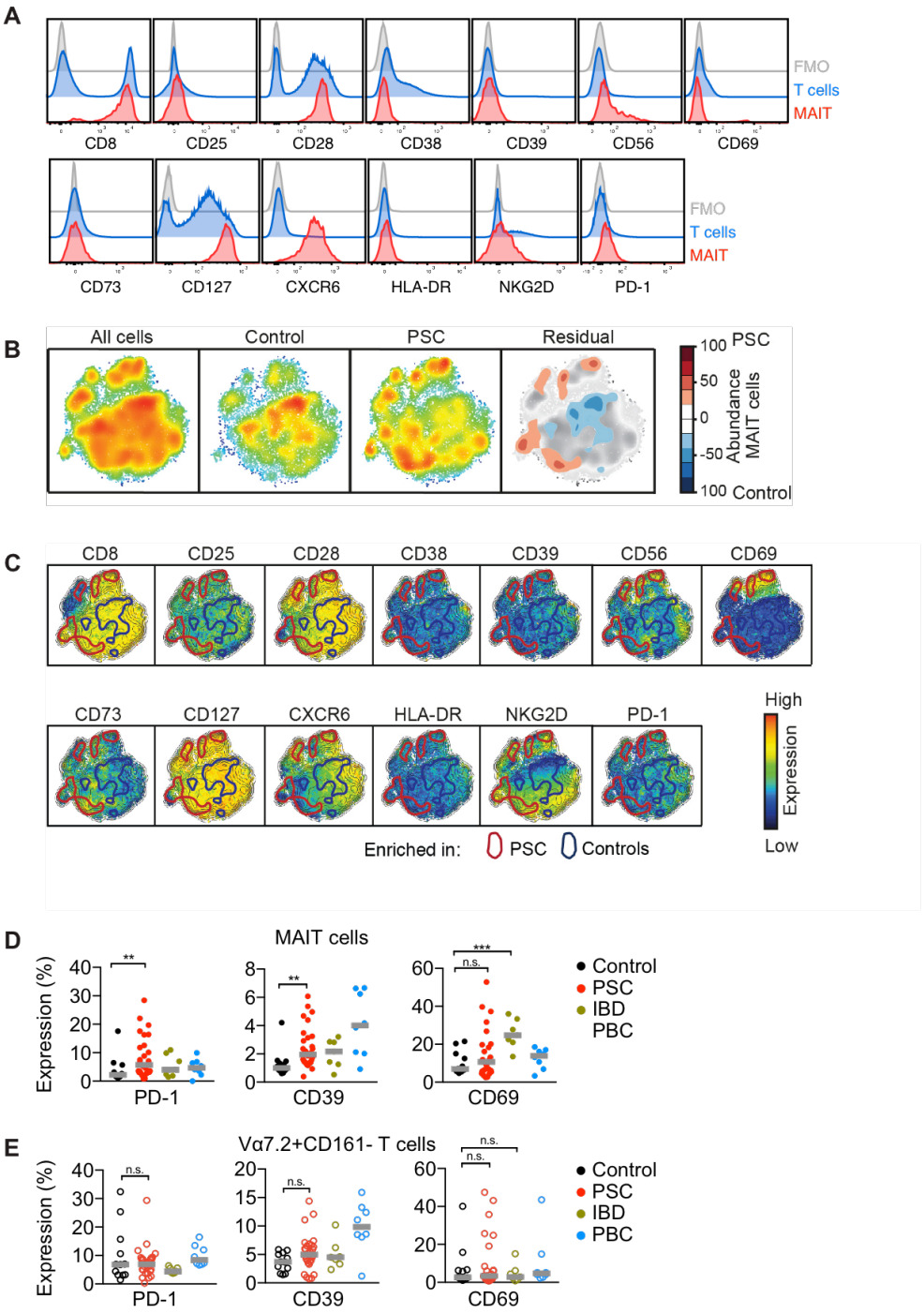
Next, we performed a detailed phenotypic characterization of the residual peripheral blood MAIT cells in PSC patients. To this end, we simultaneously analyzed the expression of 13 surface receptors on MAIT cells (Figure 12A). Stochastic Neighbour Embedding (SNE) was used for the downstream analysis. SNE is a dimensionality reduction method that generates two-dimensional representations of multi-dimensional data while preserving single-cell resolution. This method has proven well suited for analysis of complex flow cytometry data (214, 216). In brief, each sample was assigned an electronic barcode. Next, all samples were merged and analysed using Barnes-Hut SNE followed by generation of SNE maps for PSC patients and healthy controls (Figure 12B). Next, a residual plot was generated displaying the clusters of cells that varied in abundance between the two cohorts (Figure 12B). This analysis revealed substantial differences in the phenotype between MAIT cells from PSC patients as compared to healthy controls.

As a next step, to assess which phenotypic markers were contributing to the difference in phenotype, the residual plot was overlaid onto individual SNE maps showing the relative expression of each of the 13 parameters included in the analysis (Figure 12C). This revealed that cells with higher CD69, CD56, and NKG2D expression as well as lower CD28, CD127, and CXCR6 expression was present within the PSC patient clusters as compared to in cell clusters from healthy controls (Figure 12C). In a separate single-parameter conventional flow cytometry data analysis, MAIT cells from PSC-patients also presented with higher levels of

PD-1 and CD39 as compared to healthy controls (Figure 12D and Figure 12E). However, these changes were not unique to PSC but were also seen in IBD and PBC patients (Figure 9D). Interestingly, within the Vα7.2+CD161- non-MAIT cell reference population, no significant differences in phenotype were observed when comparing patients with controls suggesting the altered phenotype to be specific to MAIT cells (Figure 12E).

In summary, high-dimensional SNE analysis revealed MAIT cells to present with an activated phenotype in PSC.

Figure 12 (A) Representative histograms showing expression of the indicated markers on total T cells and MAIT cells compared to fluorescence minus one (FMO) controls. **(B)** SNE density plot of total cells subsequently subdivided into healthy controls and PSC-patients, and residual plot showing the difference between the two groups. **(C)** SNE plots showing expression of the indicated markers where cells within blue circles are more abundant in healthy controls whereas cells within the red circles are more abundant in PSC patients. **(D)** Frequency of expression for the indicated markers on MAIT cells in healthy controls and patients. **(E)** Frequency of expression for the indicated markers on Vα7.2^{+}CD161^{-} T cells in healthy controls and patients.



4.4.3 MAIT cell function in response to E. coli in PSC

Following a detailed phenotypic characterization of MAIT cells, we set out to explore their functional capacity upon stimulation with bacteria and cytokines. MAIT cells were assessed for degranulation capacity (CD107a and upregulation of granzyme B), cytokine production (TNF, IFNγ, and IL-17), as well as upregulation of activation markers (CD69 and CD25) in response to fixed E. coli or stimulation with IL-12 and IL-18 (Figure 13A). The measured responses to E. coli stimulation were mainly MR1-dependent (data not shown). An impaired MAIT cell response to E. coli stimulation was observed in the PSC-patients with reduced levels of CD107a, TNF, IFNγ, and CD69 (Figure 13B). This was also the case for the investigated disease control cohorts (Figure 13B). Compared to E. coli stimulation, only a minor impairment in MAIT cell function was noted after IL-12 and IL-18 stimulation with significantly lower levels of TNF (Figure 13C). Only very low albeit consistently detectable levels of IL-17 were observed upon the two stimulations but with no apparent changes in patients compared to controls (data not shown).

Taken together, our results indicate that MAIT cells in PSC patients have impaired MR1-dependent functional responses. In line with the profound depletion of MAIT cells and the abnormal phenotype, this is however not specific to PSC but also seen for other inflammatory conditions such as IBD and PBC.

4.4.4 Characterization of MAIT cells in human bile duct

MAIT cells are enriched in the human liver (217) and have been shown to localize in portal tracts and around bile ducts (92). Furthermore, cholangiocytes can present antigens and activate MAIT cells via MR1 (92). To assess MAIT cells present in the biliary epithelium we established a novel method of acquiring cells from biliary brush samples. Samples were obtained from PSC patients and control patients referred to endoscopic retrograde cholangiopancreatography (ERCP), the clinical procedure used to access the bile duct via the duodenum. Clinical characteristics of the investigated subjects are presented in Table 14. MAIT cells could readily be identified when such samples were analyzed (Figure 14A). The proportion of MAIT cells in biliary brush samples was elevated four-fold as compared to matched peripheral blood (Figure 14B). In contrast to the loss of MAIT cells observed in peripheral blood of PSC patients (Figure 11B), no changes in MAIT cell frequencies nor in absolute numbers were noted in biliary brush samples from PSC patients compared to non-PSC patient controls (Fig 14C and D). In summary, these data show that MAIT cells were highly enriched in bile duct tissue compared to peripheral blood, indicating that MAIT cells are retained at the site of inflammation in PSC.

Figure 13 (A) Representative concatenated FACS plots showing CD107a, granzyme B (GzmB), TNF, IFNγ, CD69, and CD25 expression following co-culture with *E. coli* or after IL-12+IL-18 stimulation. **(B)** Summary of functional data following *E. Coli* stimulation showing expression frequencies or mean fluorescence intensity (MFI) for indicated markers and sample cohorts. **(C)** Summary of functional data following IL-12+IL-18 stimulation showing expression frequencies or MFI for indicated markers and sample cohorts.

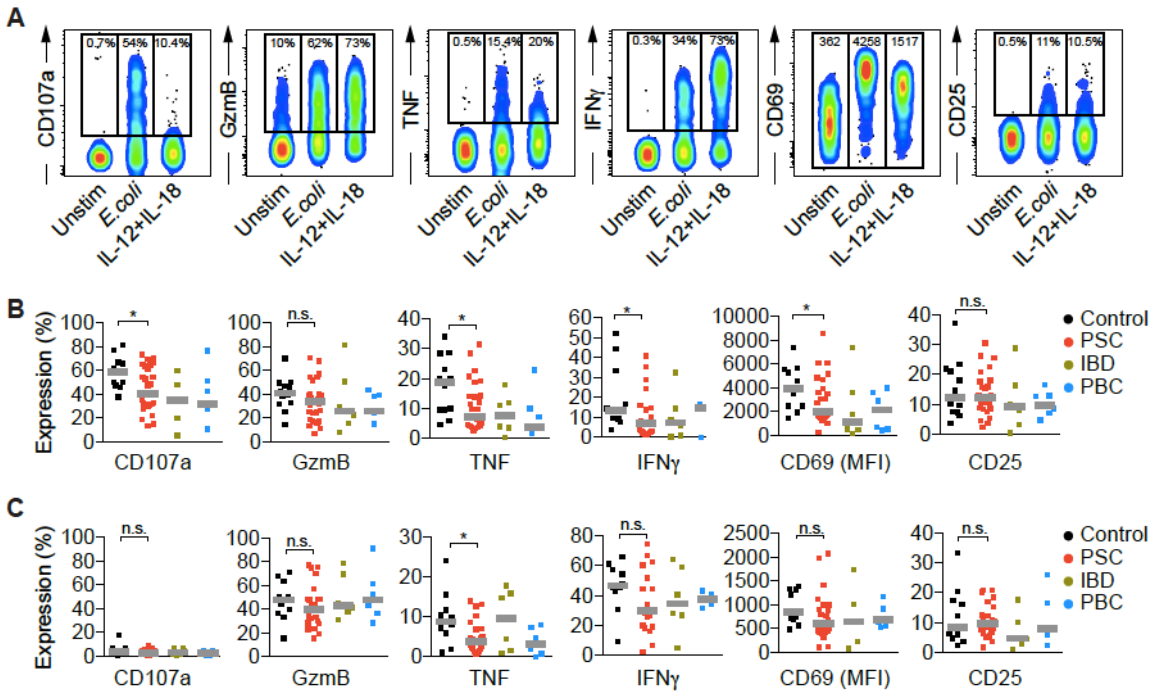


Figure 14 **(A)** Representative FACS plots of MAIT cells in matched PBMC and biliary brush samples. **(B)** Frequency of MAIT cells of total T cells in matched PBMC and biliary brush samples. **(C)** Frequency of bile duct MAIT cells out of total T cells comparing non-PSC controls and PSC patients. **(D)** Absolute number of MAIT cells obtained per biliary brush sample comparing non-PSC controls and PSC patients.

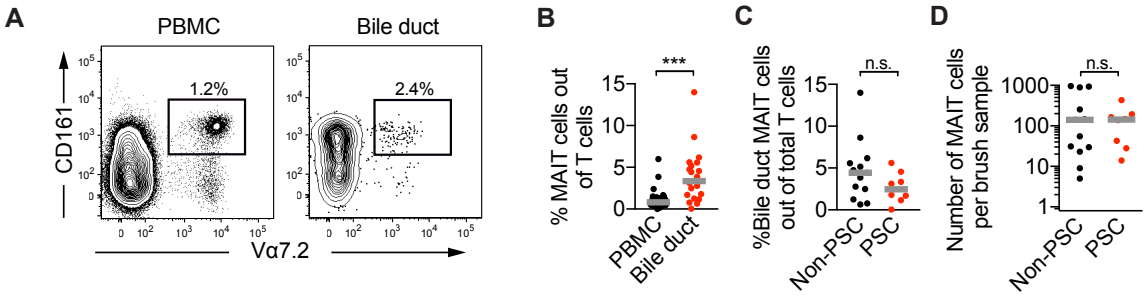


Table 14 Clinical characteristics of PSC patients and controls from which MAIT cells in bile duct were analyzed (biliary brush cohort).

	PSC (n=8)	**Controls (n=12)**
Male sex, %	38% (3/8)	58% (7/12)
Age, years (median, range)	37 (26-46)	60 (53-81)
PSC duration, years (median, range)	4 (0-24)	NA
IBD, % **Ulcerative colitis** **Mb Crohn** **Indeterminate colitis**	88% (7/8) 86% (6/7) 0% (0/7) 14% (1/7)	0% (0/12)
Bilirubin, µmol/L (median, range)	11.5 (5-96)	8.5 (3-322)
ALP, µkat/L (median, range) **% >ULN**	3.9 (0.6-7.7) 75% (6/8)	1.6 (0.7-48.1) 25% (3/12)

5 GENERAL DISCUSSION

The general objective of this thesis was to explore different aspects on biliary strictures in PSC. So far, there is a paucity of knowledge regarding mechanisms involved in the chronic injury of the bile ducts in this disease. Once strictures are established in the large bile ducts, endoscopic treatment is often needed to relieve symptoms. However, there is a lack of evidence that endoscopic treatment will benefit the long-term outcome of patients. The downside of intervention, risks and adverse effects, is also insufficiently understood in PSC. Diagnosis of CCA in PSC also remains a challenge. Because of diagnostic methods with low accuracy, PSC patients are highly likely to be subjected to repeated invasive examinations in order to detect or rule out the presence of malignancy. Consequently, in the setting of PSC, we aimed to: Investigate and describe the risks of ERCP (Paper I). Evaluate the feasibility of SOC, an endoscopic technique for detecting CCA (Paper II). Evaluate the diagnostic performance of biliary brush cytology with stepwise use of FISH (Paper III). And finally, returning to the pathogenic aspect of the bile duct stricture, explore the potential role of MAIT cells in PSC and establish a novel method for assessment of MAIT cells in the biliary epithelium (Paper IV).

5.1 PSC AND THE RISK OF ERCP-RELATED ADVERSE EVENTS

In our study (Paper I), we used data from a quality register with prospectively registered data on procedure indications and maneuvers. We found that adverse events were frequent in the PSC group with 7.8% and 7.1% of PSC patients developing PEP and cholangitis respectively. In total, 18.4% of PSC patients had at least one adverse event registered. When comparing to non-PSC patients, this corresponds to a more than two-fold increase in risk for overall adverse events and PEP, and an almost fourfold increase in risk for cholangitis. Furthermore, we used logistic regression to develop predictive models for adverse events. In these models, PSC remained an independent risk factor for adverse events overall, PEP, cholangitis and extravasation of contrast.

Previous studies examining the risk of adverse events following ERCP in PSC has reported a wide range of complications rates, 1.8%-17% (149, 154, 156, 166-170, 218-220). Although not consistent, the majority of studies have found a higher risk of cholangitis (0.6%-8%) and PEP (1.2%-7%) in PSC than in other patient groups (162). The reason for the wide range of reported frequencies of adverse events is not known. However, most studies are retrospective series evaluating stricture treatment. This might, for example, introduce selection bias since a prerequisite of inclusion would be successful cannulation thereby excluding patients with failed cannulation. Information bias, with unplanned data collection, is also a known drawback of the retrospective design. The small size of study cohorts is also a limitation in many studies. Furthermore, predisposing patient factors for adverse events and their relation to procedural risk factors (e.g. difficult cannulation and precut sphincterotomy) are only studied to a limited extent in PSC.

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5.1.1 Post-ERCP pancreatitis in PSC

Risk factors for PEP have been identified by several studies (162-165, 208). Patient related factors include young age, female sex and the presence of a previous sphincterotomy. It is difficult to determine how these general factors affect the risk in PSC on a group-level. In our study, we found PSC patients more likely to be male, younger and a previous sphincterotomy was prevalent in 18% of PSC patients compared to 8% in the non-PSC group. Procedure related factors such as difficult cannulation (prolonged papilla contact, precut-sphincterotomy), guide-wire cannulation of the pancreatic duct, sphincterotomy, cholangioscopy and therapeutic procedures (dilatation, stenting) are also associated with increased of PEP. These risk factors for PEP were mainly confirmed by our data. The distribution of procedure-related factors between PSC and non-PSC patients was unequal. For example sphincterotomy and stenting were more common interventions in non-PSC patients but other procedures such as biliary brushing and balloon dilatation were more likely to be performed in the PSC group. However, when adjusting for known risk factors, PSC remained as an independent risk factor for PEP in the predictive model, aOR 2.02 (95% CI 1.41-2.88).

The high frequency of PEP in PSC (7.8%) in our study is in line data from a large retrospective patient series from Finland by Ismail et al, including 441 ERCP-procedures in PSC patients, in which the risk of PEP was 7.0% (168). Several risk factors for PEP in PSC were identified in this study. Female sex and a guide wire in the pancreatic duct were associated with a higher risk of PEP (OR 2.6 and OR 8.2 respectively). Interestingly a previous biliary sphincterotomy was shown to be a protective factor (OR 0.28). This was also suggested by data in our study where all cases of PEP in the PSC-group had a naïve papilla (n=26). We however deemed the PSC group to small to allow for subgroup-analysis of risk factors.

The reason why PSC patients seem to have a high risk of PEP can be discussed. The notion that PSC patients are predisposed to difficult cannulation is to some extent supported by the study by Ismail. Despite that 37.8% of all procedures were done in patients with a previous sphincterotomy, access techniques such as pancreatic sphincterotomy and pre-cut sphincterotomy were used to a large extent (11.8% and 2.5%). Unfortunately, factors concerning cannulation difficulties were not registered in the national registry GallRiks between 2007 and 2009 and therefore not possible to assess in our study. Intriguingly, PSC seems to affect the pancreatic duct in some patients (221, 222). Sclerotic disease and early signs of pancreatic changes associated with extrahepatic bile duct sclerosis can be detected by MRI in a large proportion of patients with PSC (24%) (222). Hypothetically, the vulnerability of the pancreas may therefore be increased due to chronic inflammation and the response to injury more pronounced than in patients with a normal pancreas.

5.1.2 Post-ERCP cholangitis in PSC

The high risk of cholangitis after ERCP in PSC found in our study has previously been reported in several studies (149, 166, 167, 169, 219, 220). Bangarulingam et al compared the risk of adverse events in PSC (n=168) and non-PSC (n=981). Despite the use of prophylactic antibiotics, the incidence of cholangitis was significantly higher among PSC patients (4% vs. 0.2%) (166). This increased risk is likely related to inherent conditions of PSC with multiple strictures and impaired biliary drainage. Instrumentation then further facilitates colonization of gut flora with subsequent cholangitis. The registered use of prophylactic antibiotics among PSC patients in our cohort was surprisingly low, only 49%, despite the known risks in this patient group. However, we believe that this finding may reflect underreporting and/or inconsistency in the registry and a subgroup analysis did not suggest any association between prophylactic antibiotics and cholangitis in PSC patients.

5.1.3 Extravasation of contrast

The risk for extravasation of contrast was increased in PSC patients and occurred in 5.7% patients. Extravasation of contrast during ERCP is in most cases caused by a guide-wire perforation of the bile duct (223). This is a benign and self-limiting event in general (223). Moderate to severe perforations on the other hand are separately registered in GallRiks and only occurred in one PSC patient (0.7%). The increased risk for extravasation of contrast in PSC is likely caused by the strictured and narrow bile ducts that make navigation of the guide wire more difficult. The clinical impact of this risk is uncertain. Wire perforations are usually not associated with severe symptoms or outcome but may interfere with ERCP procedure and lead to premature termination and, in some cases, stenting of the affected bile duct. This would in turn generate prolonged hospital stay and a further need of ERCPs.

5.1.4 Strengths and limitations

A major strength of this study is the prospectively collected data registered in GallRiks. The use of appointed coordinators and validation of the registry further improves quality of data, allowing for a large number of variables with high reliability. In this study, we also validated that the indication “PSC or suspicion of PSC” actually represented a diagnosis of PSC. Nevertheless, misclassification in the opposite direction was not addressed in our validation. Most likely some PSC patients have been categorized as non-PSC patients with indications such as obstructive jaundice or malignancy. The effect of this is however expected to be small, diluted by the size of the control cohort.

PEP prophylaxis includes the use of rectal non-steroidal anti-inflammatory drugs (NSAIDs) and pancreatic stent placement (224). A limitation in our study was that the use of prevention strategies for PEP was not registered in GallRiks during the period 2007-2009. The impact of these measures has not specifically been assessed in PSC. Therefore, information on its use would have had additional value in this aspect.

5.2 DETECTING MALIGNANCY IN PSC

Diagnosing early CCA by non-invasive techniques generally lacks specificity. Bile duct sampling by ERCP has the ability to provide important diagnostic information in PSC patients with suspected features of biliary malignancy such as clinical deterioration, elevated levels of CA 19-9 or suspicious strictures seen on imaging (47). In two studies we evaluated two different invasive diagnostic methods for the detection CCA in PSC (Paper II and III). In our prospective study of the clinical utility of SOC in PSC we evaluated technical feasibility, safety and diagnostic accuracy of targeted sampling (Paper II). We found that SOC could successfully be used in almost all (96%) included patients. In a retrospective diagnostic study, we evaluated the diagnostic performance of biliary brush cytology with sequential use FISH in equivocal cases (Paper III). We show that the diagnostic algorithm used had higher sensitivity (80%) than expected from routine cytology alone with an acceptable level of specificity (96%). Diagnostic accuracy (i.e. proportion of correctly diagnosed patients) was 95% (197/208).

5.2.1 SOC

5.2.1.1 Technical feasibility

The main possible advantage of SOC in PSC is that it offers direct visualization of the bile duct and enables sampling of suspicious strictures with targeted biopsies. SOC has been evaluated in several case series of non-PSC patients with indeterminate strictures (205, 206, 225, 226). In comparison to brush cytology alone SOC with targeted biopsies is reported to increase the sensitivity (77%-82%) for detecting biliary malignancy (206, 225). The benefit of SOC in PSC is however not obvious for two main reasons. First, the altered morphology of the biliary tree with narrow ducts makes cannulation with the cholangioscope technically difficult (227) and the use of cholangioscopy increase the risks of adverse events (228). Second, the inflammatory and fibrotic bile ducts of PSC patients make it difficult to visually discriminate them from malignant lesions (229). Despite these obstacles, we showed that SOC was technically feasible with successful cannulation, adequate visualization and sampling of suspected lesions in 45/47 (96%) of patients. This is line with what Siiki et al reported in a smaller series of PSC patients (n=11) evaluated with SOC in which all examinations were technically successful (230).

5.2.1.2 Sampling adequacy and diagnostic accuracy

A limitation of SOC is the small amount of tissue acquired by the mini-forceps used for sampling (225). Although not defined in the study protocol, at least four separate biopsies were taken per evaluated stricture. This rendered a sampling adequacy of 95% (21/22).

In general, the visual quality of SOC did not allow for discrimination between inflammation and premalignant lesions. Only one patient with malignancy was identified by endoscopic appearance. In all strictures categorized as suspicious for cancer (3/3) by macroscopic features, sampling results revealed no malignancy or dysplastic changes. In studies evaluating

SOC in other patient categories, visual assessment as been reported to be the most sensitive method to detect CCA (sensitivity 95%, specificity 75%) (206). In our series we used the first generation fiber-optic Spyglass system with image quality often suboptimal and easily impaired by mucus, blood or PSC alterations in the bile duct. Since the study period, a newer system has been developed with a digital image solution (SpyGlass DS; Boston Scientific) and higher image quality. However, even with instruments with superior image quality to the first generation Spyglass system the detection of dysplasia seems difficult in PSC. In a case-series of 30 PSC patients, high-resolution per-oral video cholangioscopy with narrow-band imaging (NBI) was evaluated for detection of CCA and dysplasia (231). With this method, CCA and tumor margins could be detected, but visual assessment of the bile duct mucosa did not increase the detection of dysplasia.

Three patients were diagnosed with CCA during follow-up. The first patient was diagnosed at inclusion and had endoscopic appearance, biopsies and brush cytology results positive for malignancy. The two remaining patients were diagnosed after 17 months with and intrahepatic CCA and after 35 months with a hilar CCA. We included these patients when evaluating diagnostic accuracy for SOC-guided sampling, although this approach can be criticized. Sensitivity, specificity, PPV, NPV and accuracy were 33% (95% CI 1-91%), 100% (95% CI 92-100%), 100% (95% CI, 3-100%), 95% (95% CI 85-99%) and 96% (95% CI 85-100%). The few number of events however precludes any firm conclusions based on these findings.

5.2.1.3 Adverse events

The frequency of previously reported ERCP-related adverse events in PSC vary considerably (232). We found a relatively high risk of procedure-related adverse events in this patient series. In 7/47 patients (14%) complications occurred. In six cases, the adverse events were classified as mild with little impact or prolonged hospital admission no longer than 3 days. One case of cholangitis was classified as moderate. Although the few number of patients limits conclusions we argue that risks should be carefully weighed when evaluating patients with this invasive technique.

5.2.1.4 Additional technical value

We also tried to evaluate possible technical advantages associated with SOC. First, one possible advantage is guidance through visualization of the bile duct to pass the guide-wire through narrow strictures. This was found to be the case in 4/45 (9%) patients. Second, it has previously been described that SOC increases the detection and subsequent removal of bile duct stones in PSC (229). In 12/45 (27%) patients in our series biliary stones were detected and removed in 11 of these cases. The clinical implication of these possible advantages remains to be determined. To access and sample suspicious lesions in PSC patients can be technically demanding. SOC could, in this setting, offer a technique to reach and sample otherwise inaccessible strictures when necessary. Whether increased detection and removal

of biliary stones in PSC is beneficial or not is not known. These hypothetical advantages deserve attention in future studies on the subject.

5.2.1.5 Strengths and Limitations

The main strength of this study is the prospective design that allows for a robust evaluation of success rates and adverse events. However, given the small sample size results should be interpreted with caution. Also the limited number of patients with malignancy precludes any meaningful evaluation of the diagnostic accuracy of SOC with targeted biopsies in this patient-series.

5.2.2 Biliary brush cytology and FISH

In this retrospective study (Paper III), we evaluated the diagnostic performance of brush cytology with sequential use of FISH in equivocal cases for detection of CCA and biliary dysplasia in patients with PSC. We showed that a correct diagnosis could be made in 95% (197/208) of cases in this relatively unselected cohort of PSC patients with a clinical indication for ERCP. We also demonstrated that sequential use of FISH in equivocal cases shows a higher sensitivity (80%), than previously reported results of routine cytology, while an acceptable level of specificity (96%) was maintained (192). Furthermore, we found that FISH was highly sensitive (100%) for the detection of CCA in PSC patients with no evidence of malignancy on prior imaging and that false positive results correlated to biliary dysplasia in explanted livers.

5.2.2.1 The positive predictive value of FISH

We believe that the present study, complements the current data on the value of FISH analysis in PSC. Previous studies in the field have focused on evaluation of the risk for development of CCA in patients with a positive FISH analysis using a longer follow-up time. In one study of 102 PSC patients with no definite signs of CCA on imaging and equivocal cytology results, 29% was diagnosed with CCA within 2 years. The risk of CCA was increased more than eight times in cases with a positive FISH (HR 8.70 (95% CI 3.79–19.99). In another study of 235 PSC patients, in which patients were followed up to 34 months, the sensitivity of FISH for detection of later CCA development was shown to be 46% and the specificity 88%. At Karolinska University Hospital, as well as in all Nordic liver transplant centers, biliary dysplasia is an indication for LTx in PSC. This transplant setting, in which patients with HGD are offered LTx, has allowed us to evaluate the diagnostic accuracy of biliary brush cytology with FISH in a relatively short perspective (within 12 months). We therefore chose a diagnostic study design in contrast with multiple previous studies evaluating FISH as a risk factor for developing CCA at long-term follow-up (196, 199, 201, 202, 233). The diagnostic perspective in the present study gives further guidance how to interpret brush cytology in PSC patients.

As anticipated, applying FISH in equivocal cases of routine brush cytology showed a higher sensitivity than previously published results of routine cytology (192), but at the cost of

decreased specificity for identifying established CCA. The PPV of a brush positive for either malignant cells or FISH was 60% in our study. This may in part be explained by previously published results showing that the genetic alterations detected by FISH are not specific for CCA but also present in preneoplastic changes. In a study of by DeHaan et al., biliary dysplasia in liver resections and explanted livers from PSC patients, demonstrated either FISH polysomy or homozygous 9p21 loss in all specimens (5/5) (234). Thus, it is likely that, at least partly, the positive findings of FISH in this study are explained by biliary dysplasia.

When including HGD and both HGD and LGD as positive endpoints, PPV increased from 60% for CCA only to 75% and 90%. Although the number of patients with dysplasia was low in the present study, all patients with HGD in explanted livers (3/3) had a brush cytology positive for FISH. Previously published data strongly supports that CCA develops in areas with dysplastic lesions in PSC, but the time and course of this process is not known (186). In addition, PSC patients positive for FISH polysomy, either in equivocal cytology or in multifocal or serial samples, are highly likely to develop CCA (196, 199, 201, 202). Chromosomal aberrations detected by FISH may itself indicate a higher risk for malignant transformation than histologically identified dysplasia. Although probable, it is, however, difficult to obtain figures supporting this assumption. Risk stratification of PSC patients according to FISH status might be a rational approach although we note that cancer was already prevalent in 58% of patients with equivocal brush cytology with positive FISH and a negative imaging (i.e. no definite malignancy).

5.2.2.2 The negative predictive value of FISH

A high NPV of 98% was reached using FISH in equivocal cases. In clinical practise, it is important to rule out the presence of CCA or HGD in a significant stricture seen at imaging. Our study population includes referral cases from other regions but most patients were included from our primary catchment area. This is reflected by the relatively low prevalence of CCA of 7%, and that only two patients had obvious signs of malignancy prior to brushing. Thus, a high NPV for the entire group of patients with clinical indication for ERCP is expected. However, even in patients with equivocal cytology a negative FISH result was highly accurate for ruling out malignancy at 12 months (NPV 100%).

5.2.2.3 Strengths and limitations

Strengths of this study include the relatively large size of the study cohort covering all PSC patients who underwent biliary brushings during the study period. Also, almost all identified patients had a complete follow-up and could be included in the final analysis thus limiting selection bias. There are, however, several limitations to our study, mainly due to the retrospective design with its inherent flaws. During the study period, FISH results have been used in clinical decision-making, introducing verification bias, meaning that patients were not randomly allocated to interventions (e.g. LTx, surgical resection), which are of importance for the final diagnosis. The consequence of this partial verification would be a potential overestimation of the prevalence of early CCA and biliary dysplasia in patients with a

positive FISH. Consequently, an overestimation of the NPV would then likewise be the case since patients with a negative FISH finding might harbor undetected early CCA or HGD. Furthermore, since the results of biliary brush cytology are available to the pathologist interpreting the final histopathology (e.g. from an explanted liver) diagnostic review bias might also have affected the results in a similar fashion.

The choice of using only the index brush cytology result for calculation of the diagnostic accuracy is also debatable. The main limitation of this approach is that it also includes false negative results by sampling error, which is separate from the cytological diagnostic method itself. However, when we included results from repeated brushings the values for diagnostic accuracy did not change, although the number of cytology results with insufficient material did decrease (data not shown).

The follow-up time was restricted to 12 months. We argue that this approach is relevant for evaluation of FISH as a diagnostic test since a reduced follow-up time reflects the prevalence of CCA or dysplasia at the time of first brushing rather than progression of preneoplastic changes that might occur over time. Finally, there were a limited number of patients with CCA in our cohort, despite having a relative large overall sample size. Our results should therefore be interpreted with caution.

5.2.3 Present and future perspectives

Detection of malignant and premalignant lesions in the bile ducts of PSC patients remains a challenge. The use of FISH and SOC both provide additional diagnostic means that together and in combination with other techniques such as imaging can improve diagnostics. Studies with the aim to investigate biomarkers in serum and bile are ongoing and would further have the potential to improve diagnostics. However, the rarity of PSC makes it difficult to evaluate such markers and robust diagnostic methods cannot be expected in the near future.

5.3 MAIT CELLS IN PSC

In Paper IV we performed a detailed characterization of peripheral blood MAIT cells in PSC. We observed reduced MAIT cell frequencies, phenotypic changes consistent with activation and possibly exhaustion, as well as impaired functional responses upon MR-1 dependent and independent stimulation. Furthermore, MAIT cells were found to be enriched in the biliary epithelium as compared to peripheral blood, and comparable to MAIT cell frequencies and numbers of non-PSC controls. This suggests that MAIT cells are retained in the compartment affected by chronic inflammation in PSC.

5.3.1 Influence of inflammation and liver disease on peripheral MAIT cells

MAIT cells are enriched in the healthy human liver (217) and have been studied to some extent in different liver diseases. In chronic hepatitis C virus infection (235) as well as in alcoholic liver disease (236) MAIT cells are lost from circulation and present with functional impairment. These findings are in line with our observations in patients with PSC. Thus, it appears that a common profile of the MAIT cell population in chronic liver inflammation is a

reduction in frequency combined with functional impairment. MAIT cell depletion and dysfunction in peripheral blood have also been described for several other human diseases where the immune system is chronically activated. Conditions affecting the MAIT cell population include both single organs, for example multiple sclerosis (237), rheumatoid arthritis (238), and IBD (239), as well as more general systemic inflammatory settings, such as chronic HIV-infection (240, 241), systemic lupus erythematosus (242), and in metabolic inflammation during obesity and type 2 diabetes (243). It is currently unknown whether a common underlying mechanism is accountable for MAIT cell loss and functional impairment in these conditions or whether specific mechanisms exist in each disease.

One hypothesis that could explain the abnormal MAIT cell population is loss of gut integrity and subsequent microbial translocation. Many of the above-described conditions associate with a "leaky" gut such as chronic HCV infection (244), alcoholic liver disease (245), and chronic HIV infection (246). Increased presence of intestinal bacteria and bacterial antigens and metabolites would then lead to MAIT cell activation, and over time MAIT cell exhaustion and apoptosis as a consequence of the prolonged insult. Evidence for such a mechanism has been presented both for patients with chronic HIV infection as well as in alcoholic liver disease (245, 246). In the case of PSC, and except for the actual inflammation in the liver that could promote gut leakiness, many patients also have concomitant IBD further exacerbating the microbial translocation. Of note, we observed no significant differences regarding the MAIT cell loss and functional impairment in patients with PSC and IBD, or in patients with PSC and IBD only. The exact process by which MAIT cells become activated (and exhausted) and eventually die still remains unknown. However, it is interesting to note that removal of the chronic insult, in the case of chronic HCV via treatment with direct acting antivirals (235), does not readily lead to a reinvigoration, suggesting that the insult inflicted on the MAIT cell population during chronic inflammation might be long-lasting and perhaps irreversible.

A recent report investigated MAIT cells in humans affected by different autoimmune liver diseases, including PSC (247). Our findings are in line with this report including loss of MAIT cells from circulation, an activated (and possibly exhausted) phenotype, as well as a functional impairment. Interestingly, the authors further suggested that MAIT cells could contribute to liver fibrosis development via production of IL-17 and subsequent hepatic stellate cell (HSC) activation (247). PSC-patients are known to have increased CD4 T cell Th17-responses (73). However, in this regard, we could not detect increased MAIT cell IL-17 levels, neither upon E.coli nor cytokine stimulation.

5.3.2 MAIT cells in the biliary epithelium

PSC is a disease of extra- and intrahepatic bile ducts. Although work in previous studies have tried to localize MAIT cells within the liver parenchyma, and have reported that the cells are present in portal tracts surrounding bile ducts (92, 247), it has been challenging to specifically assess MAIT cells in bile ducts. To overcome this, we established a novel method allowing sampling of bile duct tissue during ERCP procedures for subsequent MAIT cell analysis. In

line with the fact that MAIT cells are accumulated in peripheral organs, such as the liver as well as at mucosal barriers, we here report that MAIT cells are enriched four-fold in bile ducts as compared to peripheral blood. Intriguingly, and in contrast to the circulating MAIT cell compartment, we also observed retained levels of MAIT cells in bile ducts from PSC patients as compared to controls. The specific role for MAIT cells in the pathogenesis of PSC is currently unclear. However, the possibility to assess bile duct MAIT cells will allow for future studies on this topic. The recent development of SOC with high quality visualization is also interesting in this aspect (248). The possibility to visually identify bile ducts affected by inflammation may allow for a more precise sampling and detailed assessment of the inflammatory process.

5.3.3 Limitations

Several limitations of our study have to be considered. First, we used a cross-sectional cohort with sampling at one time-point only. Second, we did not detect clear correlations between MAIT cells and parameters of disease severity. One reason for this might be that our cohort consisted of patients with relatively mild disease. Future studies should include larger patient populations and longitudinal sampling for identification of correlates between immune system function and PSC disease activity and progression. Third, and on a more technological level, we identified MAIT cells with flow cytometry using the combination of antibodies against the TCR Vα7.2 chain and CD161. Although it would have been more advantageous to instead use an MR1-tetramer, this tool was not commercially available at the time of our study. To account for this, we restricted our analysis to CD8+ or CD4-CD8- Vα7.2+CD161+ T cells, since CD4+ Vα7.2+CD161+ T cells have been shown to contain more “non-MAIT” cells (249, 250).

6 CONCLUSIONS

PSC is associated with a high risk of ERCP-related adverse events (Paper I)

PSC is an independent risk factor for ERCP-related adverse events overall and for cholangitis and pancreatitis (Paper I)

Single-operator cholangioscopy is technically feasible in PSC (Paper II)

The use of single-operator cholangioscopy to improve sensitivity of bile duct sampling for CCA in PSC could not be sufficiently evaluated in this thesis and further studies are needed to assess this. (Paper II)

Biliary brush cytology with use of FISH in equivocal cytology cases has a high diagnostic accuracy in PSC (Paper III)

A negative FISH analysis for aneuploidy in equivocal cytology cases has a very high negative predictive value for cholangiocarcinoma (Paper III)

PSC is associated with an altered MAIT cell population in peripheral blood with reduced frequencies, an activated phenotype and impaired function (Paper IV)

Alterations of the MAIT cell population in PSC are similar do those seen in patients with ulcerative colitis and primary biliary cholangitis (Paper IV)

Using biliary brush samples to assess MAIT cells in the biliary epithelium is feasible (Paper IV)

MAIT cells are retained in the biliary epithelium in PSC (Paper IV)

7 POPULÄRVETENSKAPLIG SAMMANFATTNING

Primär skleroserande kolangit (PSC) är en ovanlig men allvarlig leversjukdom med dålig prognos kopplad till en stor risk för utveckling av både levercirros (skrumplever) och gallgångscancer. Det som kännetecknar sjukdomen är en kronisk inflammation i gallgångarna i och utanför levern. Inflammationen leder till ärrbildning och förträngningar (strikturer) som förhindrar flödet av galla. Detta leder till utveckling av levercirros och sviktande funktion hos levern. PSC är starkt kopplat till inflammatorisk tarmsjukdom som finns hos 80 % av patienterna. Sjukdomen är ovanlig, i Norden uppskattas den förekomma hos 10 individer per 100 000 invånare. Det som har kommit att radikalt förändra prognosen vid PSC är utvecklingen av levertransplantation som behandling under början av 1980-talet. Innan dess dog en stor andel patienterna av leversvikt och andra komplikationer. Sjukdomsförloppet varierar stort men efter ungefär 20 år är hälften av patienterna antingen transplanterade eller döda. Trots att PSC är ovanligt är det den vanligaste orsaken till levertransplantation i Sverige och Norden. Det finns en starkt ökad risk för både tjocktarmscancer och cancer i gallgångarna (kolangiocarcinom) vid PSC. Ungefär 10 % av alla patienter utvecklar kolangiocarcinom och denna cancerform har mycket dålig prognos om man inte kan ge botande behandling i tidigt skede.

Under de senaste decennierna har forskning gett en ökad förståelse för PSC. Kartläggning av genetiska faktorer har visat en stark koppling mellan sjukdomen och gener inblandade i immunförsvarets utveckling och reglering. Samtidigt finns en stark koppling till andra så kallade autoimmuna sjukdomar som typ 1 diabetes och glutenintolerans. Även om PSC är ovanlig så verkar den öka i befolkningen över tid, precis som andra autoimmuna sjukdomar. Orsaken till det är inte känd men olika teorier finns om hur miljöfaktorer som ökad hygien (minskad exponering för mikroorganismer) och förändrad kost kan påverka risken. Nya röntgentekniker, förförallt magnetkameraundersökning (MR), har också gjort det lättare att hitta och diagnosticera patienter. Trots framsteg finns fortfarande ingen medicinsk behandling som påverkar sjukdomsförloppet. Inte heller har vi någon klar bild av hur sjukdomen uppkommer. Gallgångscancer har fortfarande stor betydelse för den ökade dödligheten vid PSC. De diagnostiska metoder som används för att hitta cancern har i regel dålig träffsäkerhet och osäkra resultat i tidigt skede.

I denna avhandling har vi med utgångspunkt från gallvägsstrikturer vid PSC dels undersökt sjukdomsmekanismer dels studerat diagnostiska metoder för cancer i gallvägarna.

En nyckelmetod för att undersöka gallgångarna vid PSC är endoskopisk retrograd kolangiopankreatografi (ERCP). ERCP kombinerar ett videoinstrument (endoskop), som förs ner i tolvfingertarmen, med röntgenteknik där kontrast sprutas in i gallgången för att visualisera stenar, tumörer och andra förträngningar. Fördelen med ERCP är att man vid undersökningen också kan vidga strikturer samt ta vävnadsprover från misstänkta tumörer. Nackdelen är att det finns risk för komplikationer som kolangit (bakterieinfektion i gallvägarna) och pankreatit (inflammation i bukspottskörteln). I tre separata studier har vi utvärderat olika aspekter av denna undersökningsmetod.

I den första studien har vi kartlagt risken för komplikationer vid ERCP hos patienter med PSC. Vi utgick från ett svenskt nationellt kvalitetsregister, GallRiks, där nästan samtliga undersökningar som görs i Sverige registreras. Totalt 8932 individer varav 141 PSC-patienter ingick i studien. Vi fann att komplikationer vid ERCP var mer än dubbelt så vanligt hos patienter med PSC jämfört med andra patienter. Särskilt stor var risken för kolangit och pankreatit.

Kolangioskopi är en endoskopisk metod för att titta in i gallgångarna och ta vävnadsprover från misstänkt cancer. I studie II kunde vi visa att kolangioskopi med vävnadsprover var tekniskt genomförbart vid PSC. Större studier behövs för att påvisa om metoden tillför nytta vid cancerdiagnostik i denna patientgrupp.

Borstcytologi (cellprovtagning) är en annan metod för att påvisa cellförändringar och cancer i gallvägarna men resultaten är ofta svårtolkade och osäkra. I studie III utvärderade vi om denna metod kan förbättras genom att också analysera kromosomförändringar som kan finnas hos cancerceller. Vi kunde i denna studie visa att detta gav en ökad säkerhet i diagnostiken med en ökad känslighet för att upptäcka gallvägscancer.

Så kallade mucosal associated invariant T-cells (MAIT-celler) är en grupp av immunceller som försvarar kroppen mot angrepp av mikroorganismer i framförallt slemhinnor. Dessa celler är i hög grad ansamlade också i levern men deras funktion där är i stora delar okänd. I den fjärde studien har vi undersökt och karaktäriserat MAIT-celler i blodet hos patienter med PSC och jämfört med andra patientgrupper (med inflammatorisk tarmsjukdom och annan kronisk leversjukdom) samt friska individer. Vi kunde i denna studie visa att MAIT-celler hos PSC patienter i hög grad skiljer sig från de som finns hos friska individer. I blod uppvisar dessa celler tecken på att vara aktiverade och ”utmattade”. Vi har också utvärderat MAIT-cellers förmåga att döda infekterade celler och rekrytera andra delar av immunförsvaret. Dessa funktioner visade sig vara nedsatta vid PSC jämfört med friska individer. MAIT-cellernas profil vid PSC (aktivering, utmattning och nedsatt funktion) är dock inte specifik för sjukdomen utan liknar den hos patienter med inflammatorisk tarmsjukdom eller annan leversjukdom. I denna studie utvecklade vi också en metod för att analysera MAIT-celler från cellprover tagna i gallvägarna. Vi fann att MAIT-celler är anrikade i denna del av kroppen jämfört med blod både vid PSC och hos andra patientgrupper.

Sammanfattningsvis ger resultaten från denna avhandling insikt i flera viktiga aspekter av det kliniska omhändertagandet av patienter med PSC. Vi har visat att risken för komplikationer vid ERCP hos denna patientgrupp är stor. Vi visar också hur borstcytologi kan förbättras för mer exakt diagnostik av gallvägscancer. Vidare fann vi att kolangioskopi har en potentiell roll i denna diagnostik men för att påvisa nytta behövs större studier. Vi har också detaljerat beskrivit MAIT-celler vid PSC. Slutligen har vi med en nyutvecklad metod påvisat dessa celler i gallvägarna hos individer med och utan PSC.

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9 REFERENCES

1. Delbet P. Retrecssement du choledoque : Choleocysto-duodenostomie. Bull Mem Soc Nat Chir. 1924 1924;50:1144-6.

2. Wiesner RH, LaRusso NF. Clinicopathologic features of the syndrome of primary sclerosing cholangitis. Gastroenterology. 1980 Aug;79(2):200-6. PubMed PMID: 7399227. Epub 1980/08/01. eng.

3. Schrumpf E, Elgjo K, Fausa O, Gjone E, Kolmannskog F, Ritland S. Sclerosing Cholangitis in Ulcerative Colitis. Scandinavian Journal of Gastroenterology. 1980 1980/09/01;15(6):689-97.

4. Chapman RW, Arborgh BA, Rhodes JM, Summerfield JA, Dick R, Scheuer PJ, et al. Primary sclerosing cholangitis: a review of its clinical features, cholangiography, and hepatic histology. Gut. 1980 1980-10-01 00:00:00;21:870-7.

5. Mendes FD, Jorgensen R, Keach J, Katzmann JA, Smyrk T, Donlinger J, et al. Elevated serum IgG4 concentration in patients with primary sclerosing cholangitis. Am J Gastroenterol. 2006 Sep;101(9):2070-5. PubMed PMID: 16879434. Epub 2006/08/02. eng.

6. Perdigoto R, Carpenter HA, Czaja AJ. Frequency and significance of chronic ulcerative colitis in severe corticosteroid-treated autoimmune hepatitis. Journal of hepatology. 1992 Mar;14(2-3):325-31. PubMed PMID: 1500696. Epub 1992/03/01. eng.

7. Wee A, Ludwig J. Pericholangitis in chronic ulcerative colitis: primary sclerosing cholangitis of the small bile ducts? Annals of internal medicine. 1985 May;102(5):581-7. PubMed PMID: 3985511. Epub 1985/05/01. eng.

8. Broome U, Lindberg G, Lofberg R. Primary sclerosing cholangitis in ulcerative colitis--a risk factor for the development of dysplasia and DNA aneuploidy? Gastroenterology. 1992 Jun;102(6):1877-80. PubMed PMID: 1587406. Epub 1992/06/01. eng.

9. Wee A, Ludwig J, Coffey RJ, Jr., LaRusso NF, Wiesner RH. Hepatobiliary carcinoma associated with primary sclerosing cholangitis and chronic ulcerative colitis. Hum Pathol. 1985 Jul;16(7):719-26. PubMed PMID: 4007848. Epub 1985/07/01. eng.

10. Boonstra K, Weersma RK, van Erpecum KJ, Rauws EA, Spanier BW, Poen AC, et al. Population-based epidemiology, malignancy risk, and outcome of primary sclerosing cholangitis. Hepatology (Baltimore, Md). 2013 Dec;58(6):2045-55. PubMed PMID: 23775876.

11. Broomé U, Olsson R, Lööf L, Bodemar G, Hultcrantz R, Danielsson A, et al. Natural history and prognostic factors in 305 Swedish patients with primary sclerosing cholangitis. Gut. 1996 Apr;38(4):610-5. PubMed PMID: 8707097. Pubmed Central PMCID: PMC1383124. eng.

12. Aadland E, Schrumpf E, Fausa O, Elgjo K, Heilo A, Aakhus T, et al. Primary sclerosing cholangitis: a long-term follow-up study. Scand J Gastroenterol. 1987 Aug;22(6):655-64. PubMed PMID: 3659828. Epub 1987/08/01. eng.

13. Lerut J, Demetris AJ, Stieber AC, Marsh JW, Gordon RD, Esquivel CO, et al. Intrahepatic bile duct strictures after human orthotopic liver transplantation. Recurrence of primary sclerosing cholangitis or unusual presentation of allograft rejection? Transpl Int.

1988 Oct;1(3):127-30. PubMed PMID: 3075471. Pubmed Central PMCID: PMC2979302. Epub 1988/10/01. eng.

14. National Institutes of Health Consensus Development Conference Statement: liver transplantation--June 20-23, 1983. Hepatology (Baltimore, Md). 1984 Jan-Feb;4(1 Suppl):107S-10S. PubMed PMID: 6363254. Epub 1984/01/01. eng.

15. Leader M, Campbell A. Rare diseases: Europe's challenges. Scrip Drug Market Developments. 2008;19(5):17-20.

16. Molodecky NA, Kareemi H, Parab R, Barkema HW, Quan H, Myers RP, et al. Incidence of primary sclerosing cholangitis: a systematic review and meta-analysis. Hepatology (Baltimore, Md). 2011 May;53(5):1590-9. PubMed PMID: 21351115. Epub 2011/02/26. eng.

17. Tanaka A, Takikawa H. Geoepidemiology of primary sclerosing cholangitis: a critical review. Journal of autoimmunity. 2013 Oct;46:35-40. PubMed PMID: 23932346. Epub 2013/08/13. eng.

18. Lindkvist B, Benito de Valle M, Gullberg B, Bjornsson E. Incidence and prevalence of primary sclerosing cholangitis in a defined adult population in Sweden. Hepatology (Baltimore, Md). 2010 Aug;52(2):571-7. PubMed PMID: 20683956. Epub 2010/08/05. eng.

19. Bernstein CN. Review article: changes in the epidemiology of inflammatory bowel disease-clues for aetiology. Aliment Pharmacol Ther. 2017 Nov;46(10):911-9. PubMed PMID: 28949021. Epub 2017/09/28. eng.

20. Lunder AK, Hov JR, Borthne A, Gleditsch J, Johannesen G, Tveit K, et al. Prevalence of Sclerosing Cholangitis Detected by Magnetic Resonance Cholangiography in Patients With Long-term Inflammatory Bowel Disease. Gastroenterology. 2016 Oct;151(4):660-9 e4. PubMed PMID: 27342213. Epub 2016/06/28. eng.

21. Toy E, Balasubramanian S, Selmi C, Li CS, Bowlus CL. The prevalence, incidence and natural history of primary sclerosing cholangitis in an ethnically diverse population. BMC gastroenterology. 2011 Jul 18;11:83. PubMed PMID: 21767410. Pubmed Central PMCID: PMC3160402. Epub 2011/07/20. eng.

22. de Valle MB, Bjornsson E, Lindkvist B. Mortality and cancer risk related to primary sclerosing cholangitis in a Swedish population-based cohort. Liver international : official journal of the International Association for the Study of the Liver. 2012 Mar;32(3):441-8. PubMed PMID: 22098097. Epub 2011/11/22. eng.

23. Tischendorf JJ, Hecker H, Kruger M, Manns MP, Meier PN. Characterization, outcome, and prognosis in 273 patients with primary sclerosing cholangitis: A single center study. Am J Gastroenterol. 2007 Jan;102(1):107-14. PubMed PMID: 17037993. Epub 2006/10/14. eng.

24. Kaplan GG, Laupland KB, Butzner D, Urbanski SJ, Lee SS. The burden of large and small duct primary sclerosing cholangitis in adults and children: a population-based analysis. Am J Gastroenterol. 2007 May;102(5):1042-9. PubMed PMID: 17313496. Epub 2007/02/23. eng.

25. Porayko MK, Wiesner RH, LaRusso NF, Ludwig J, MacCarty RL, Steiner BL, et al. Patients with asymptomatic primary sclerosing cholangitis frequently have progressive disease. Gastroenterology. 1990 Jun;98(6):1594-602. PubMed PMID: 2338198. Epub 1990/06/01. eng.

26. Wiesner RH, Grambsch PM, Dickson ER, Ludwig J, MacCarty RL, Hunter EB, et al. Primary sclerosing cholangitis: natural history, prognostic factors and survival analysis. Hepatology (Baltimore, Md). 1989 Oct;10(4):430-6. PubMed PMID: 2777204. Epub 1989/10/01. eng.

27. Broome U, Bergquist A. Primary sclerosing cholangitis, inflammatory bowel disease, and colon cancer. Seminars in liver disease. 2006 Feb;26(1):31-41. PubMed PMID: 16496231. Epub 2006/02/24. eng.

28. Boberg KM, Aadland E, Jahnsen J, Raknerud N, Stiris M, Bell H. Incidence and prevalence of primary biliary cirrhosis, primary sclerosing cholangitis, and autoimmune hepatitis in a Norwegian population. Scand J Gastroenterol. 1998 Jan;33(1):99-103. PubMed PMID: 9489916. Epub 1998/03/07. eng.

29. Escorsell A, Pares A, Rodes J, Solis-Herruzo JA, Miras M, de la Morena E. Epidemiology of primary sclerosing cholangitis in Spain. Spanish Association for the Study of the Liver. Journal of hepatology. 1994 Nov;21(5):787-91. PubMed PMID: 7890895. Epub 1994/11/01. eng.

30. Loftus EV, Jr., Harewood GC, Loftus CG, Tremaine WJ, Harmsen WS, Zinsmeister AR, et al. PSC-IBD: a unique form of inflammatory bowel disease associated with primary sclerosing cholangitis. Gut. 2005 Jan;54(1):91-6. PubMed PMID: 15591511. Pubmed Central PMCID: PMC1774346. Epub 2004/12/14. eng.

31. Bernstein CN, Blanchard JF, Rawsthorne P, Yu N. The prevalence of extraintestinal diseases in inflammatory bowel disease: a population-based study. Am J Gastroenterol. 2001 Apr;96(4):1116-22. PubMed PMID: 11316157. Epub 2001/04/24. eng.

32. Parlak E, Kosar Y, Ulker A, Dagli U, Alkim C, Sahin B. Primary sclerosing cholangitis in patients with inflammatory bowel disease in Turkey. J Clin Gastroenterol. 2001 Oct;33(4):299-301. PubMed PMID: 11588543. Epub 2001/10/06. eng.

33. Schrumpf E, Fausa O, Kolmannskog F, Elgjo K, Ritland S, Gjone E. Sclerosing cholangitis in ulcerative colitis. A follow-up study. Scand J Gastroenterol. 1982 Jan;17(1):33-9. PubMed PMID: 7134835. Epub 1982/01/01. eng.

34. Wewer V, Gluud C, Schlichting P, Burcharth F, Binder V. Prevalence of hepatobiliary dysfunction in a regional group of patients with chronic inflammatory bowel disease. Scand J Gastroenterol. 1991 Jan;26(1):97-102. PubMed PMID: 2006402. Epub 1991/01/01. eng.

35. Lindstrom L, Lapidus A, Ost A, Bergquist A. Increased risk of colorectal cancer and dysplasia in patients with Crohn's colitis and primary sclerosing cholangitis. Dis Colon Rectum. 2011 Nov;54(11):1392-7. PubMed PMID: 21979184. Epub 2011/10/08. eng.

36. Liu JZ, Hov JR, Folseraas T, Ellinghaus E, Rushbrook SM, Doncheva NT, et al. Dense genotyping of immune-related disease regions identifies nine new risk loci for primary sclerosing cholangitis. Nat Genet. 2013 Jun;45(6):670-5. PubMed PMID: 23603763. Pubmed Central PMCID: PMC3667736. Epub 2013/04/23. eng.

37. Riley TR, Schoen RE, Lee RG, Rakela J. A case series of transplant recipients who despite immunosuppression developed inflammatory bowel disease. Am J Gastroenterol. 1997 Feb;92(2):279-82. PubMed PMID: 9040206. Epub 1997/02/01. eng.

38. Jorgensen KK, Grzyb K, Lundin KE, Clausen OP, Aamodt G, Schrumpf E, et al. Inflammatory bowel disease in patients with primary sclerosing cholangitis: clinical

characterization in liver transplanted and nontransplanted patients. Inflamm Bowel Dis. 2012 Mar;18(3):536-45. PubMed PMID: 21456044. Epub 2011/04/02. eng.

39. Lundqvist K, Broome U. Differences in colonic disease activity in patients with ulcerative colitis with and without primary sclerosing cholangitis: a case control study. Dis Colon Rectum. 1997 Apr;40(4):451-6. PubMed PMID: 9106695. Epub 1997/04/01. eng.

40. Fausa O, Schrumpf E, Elgjo K. Relationship of inflammatory bowel disease and primary sclerosing cholangitis. Seminars in liver disease. 1991 Feb;11(1):31-9. PubMed PMID: 2047887. Epub 1991/02/01. eng.

41. Weismuller TJ, Trivedi PJ, Bergquist A, Imam M, Lenzen H, Ponsioen CY, et al. Patient Age, Sex, and Inflammatory Bowel Disease Phenotype Associate With Course of Primary Sclerosing Cholangitis. Gastroenterology. 2017 Jun;152(8):1975-84 e8. PubMed PMID: 28274849. Pubmed Central PMCID: PMC5546611. Epub 2017/03/10. eng.

42. Wang R, Leong RW. Primary sclerosing cholangitis as an independent risk factor for colorectal cancer in the context of inflammatory bowel disease: a review of the literature. World J Gastroenterol. 2014 Jul 21;20(27):8783-9. PubMed PMID: 25083052. Pubmed Central PMCID: PMC4112886. Epub 2014/08/02. eng.

43. Soetikno RM, Lin OS, Heidenreich PA, Young HS, Blackstone MO. Increased risk of colorectal neoplasia in patients with primary sclerosing cholangitis and ulcerative colitis: a meta-analysis. Gastrointestinal endoscopy. 2002 Jul;56(1):48-54. PubMed PMID: 12085034. Epub 2002/06/27. eng.

44. Claessen MM, Vleggaar FP, Tytgat KM, Siersema PD, van Buuren HR. High lifetime risk of cancer in primary sclerosing cholangitis. Journal of hepatology. 2009 Jan;50(1):158-64. PubMed PMID: 19012991. Epub 2008/11/18. eng.

45. Leidenius MH, Farkkila MA, Karkkainen P, Taskinen EI, Kellokumpu IH, Hockerstedt KA. Colorectal dysplasia and carcinoma in patients with ulcerative colitis and primary sclerosing cholangitis. Scand J Gastroenterol. 1997 Jul;32(7):706-11. PubMed PMID: 9246712. Epub 1997/07/01. eng.

46. Braden B, Halliday J, Aryasingha S, Sharifi Y, Checchin D, Warren BF, et al. Risk for colorectal neoplasia in patients with colonic Crohn's disease and concomitant primary sclerosing cholangitis. Clin Gastroenterol Hepatol. 2012 Mar;10(3):303-8. PubMed PMID: 22037429. Epub 2011/11/01. eng.

47. Aabakken L, Karlsen TH, Albert J, Arvanitakis M, Chazouilleres O, Dumonceau JM, et al. Role of endoscopy in primary sclerosing cholangitis: European Society of Gastrointestinal Endoscopy (ESGE) and European Association for the Study of the Liver (EASL) Clinical Guideline. Endoscopy. 2017 Jun;49(6):588-608. PubMed PMID: 28420030. Epub 2017/04/19. eng.

48. Dave M, Elmunzer BJ, Dwamena BA, Higgins PD. Primary sclerosing cholangitis: meta-analysis of diagnostic performance of MR cholangiopancreatography. Radiology. 2010 Aug;256(2):387-96. PubMed PMID: 20656832. Epub 2010/07/27. eng.

49. European Association for the Study of the L. EASL Clinical Practice Guidelines: management of cholestatic liver diseases. Journal of hepatology. 2009 Aug;51(2):237-67. PubMed PMID: 19501929.

50. Trivedi PJ, Hirschfield GM. Review article: overlap syndromes and autoimmune liver disease. Aliment Pharmacol Ther. 2012 Sep;36(6):517-33. PubMed PMID: 22817525.

51. Hov JR, Boberg KM, Karlsen TH. Autoantibodies in primary sclerosing cholangitis. World J Gastroenterol. 2008 Jun 28;14(24):3781-91. PubMed PMID: 18609700. Pubmed Central PMCID: PMC2721433. Epub 2008/07/09. eng.

52. Boonstra K, Culver EL, de Buy Wenniger LM, van Heerde MJ, van Erpecum KJ, Poen AC, et al. Serum immunoglobulin G4 and immunoglobulin G1 for distinguishing immunoglobulin G4-associated cholangitis from primary sclerosing cholangitis. Hepatology (Baltimore, Md). 2014 May;59(5):1954-63. PubMed PMID: 24375491. Pubmed Central PMCID: PMC4489327. Epub 2014/01/01. eng.

53. Ghazale A, Chari ST, Zhang L, Smyrk TC, Takahashi N, Levy MJ, et al. Immunoglobulin G4-associated cholangitis: clinical profile and response to therapy. Gastroenterology. 2008 Mar;134(3):706-15. PubMed PMID: 18222442. Epub 2008/01/29. eng.

54. Bjornsson E, Olsson R, Bergquist A, Lindgren S, Braden B, Chapman RW, et al. The natural history of small-duct primary sclerosing cholangitis. Gastroenterology. 2008 Apr;134(4):975-80. PubMed PMID: 18395078. Epub 2008/04/09. eng.

55. EASL Clinical Practice Guidelines: Autoimmune hepatitis. Journal of hepatology. 2015 Oct;63(4):971-1004. PubMed PMID: 26341719. Epub 2015/09/06. eng.

56. Boberg KM, Chapman RW, Hirschfield GM, Lohse AW, Manns MP, Schrumpf E. Overlap syndromes: the International Autoimmune Hepatitis Group (IAIHG) position statement on a controversial issue. Journal of hepatology. 2011 Feb;54(2):374-85. PubMed PMID: 21067838. Epub 2010/11/12. eng.

57. Andersen IM, Tengesdal G, Lie BA, Boberg KM, Karlsen TH, Hov JR. Effects of coffee consumption, smoking, and hormones on risk for primary sclerosing cholangitis. Clin Gastroenterol Hepatol. 2014 Jun;12(6):1019-28. PubMed PMID: 24076415. Epub 2013/10/01. eng.

58. Eaton JE, Juran BD, Atkinson EJ, Schlicht EM, Xie X, de Andrade M, et al. A comprehensive assessment of environmental exposures among 1000 North American patients with primary sclerosing cholangitis, with and without inflammatory bowel disease. Aliment Pharmacol Ther. 2015 May;41(10):980-90. PubMed PMID: 25783671. Pubmed Central PMCID: PMC4402146. Epub 2015/03/19. eng.

59. Mitchell SA, Thyssen M, Orchard TR, Jewell DP, Fleming KA, Chapman RW. Cigarette smoking, appendectomy, and tonsillectomy as risk factors for the development of primary sclerosing cholangitis: a case control study. Gut. 2002 Oct;51(4):567-73. PubMed PMID: 12235082. Pubmed Central PMCID: PMC1773401. Epub 2002/09/18. eng.

60. van Erpecum KJ, Smits SJ, van de Meeberg PC, Linn FH, Wolfhagen FH, vanBerge-Henegouwen GP, et al. Risk of primary sclerosing cholangitis is associated with nonsmoking behavior. Gastroenterology. 1996 May;110(5):1503-6. PubMed PMID: 8613056. Epub 1996/05/01. eng.

61. Chapman RW, Varghese Z, Gaul R, Patel G, Kokinon N, Sherlock S. Association of primary sclerosing cholangitis with HLA-B8. Gut. 1983 Jan;24(1):38-41. PubMed PMID: 6600227. Pubmed Central PMCID: PMC1419910. Epub 1983/01/01. eng.

62. Bergquist A, Montgomery SM, Bahmanyar S, Olsson R, Danielsson A, Lindgren S, et al. Increased risk of primary sclerosing cholangitis and ulcerative colitis in first-degree relatives of patients with primary sclerosing cholangitis. Clin Gastroenterol Hepatol. 2008 Aug;6(8):939-43. PubMed PMID: 18674735.

63. Karlsen TH, Melum E, Franke A. The utility of genome-wide association studies in hepatology. Hepatology (Baltimore, Md). 2010 May;51(5):1833-42. PubMed PMID: 20232293. Epub 2010/03/17. eng.

64. Ji SG, Juran BD, Mucha S, Folseraas T, Jostins L, Melum E, et al. Genome-wide association study of primary sclerosing cholangitis identifies new risk loci and quantifies the genetic relationship with inflammatory bowel disease. Nat Genet. 2017 Feb;49(2):269-73. PubMed PMID: 27992413. Pubmed Central PMCID: PMC5540332. Epub 2016/12/20. eng.

65. Farh KK, Marson A, Zhu J, Kleinewietfeld M, Housley WJ, Beik S, et al. Genetic and epigenetic fine mapping of causal autoimmune disease variants. Nature. 2015 Feb 19;518(7539):337-43. PubMed PMID: 25363779. Pubmed Central PMCID: PMC4336207. Epub 2014/11/05. eng.

66. Ellinghaus D, Folseraas T, Holm K, Ellinghaus E, Melum E, Balschun T, et al. Genome-wide association analysis in primary sclerosing cholangitis and ulcerative colitis identifies risk loci at GPR35 and TCF4. Hepatology (Baltimore, Md). 2013 Sep;58(3):1074-83. PubMed PMID: 22821403. Epub 2012/07/24. eng.

67. Srivastava B, Mells GF, Cordell HJ, Muriithi A, Brown M, Ellinghaus E, et al. Fine mapping and replication of genetic risk loci in primary sclerosing cholangitis. Scand J Gastroenterol. 2012 Jul;47(7):820-6. PubMed PMID: 22554193. Epub 2012/05/05. eng.

68. Folseraas T, Melum E, Rausch P, Juran BD, Ellinghaus E, Shiryaev A, et al. Extended analysis of a genome-wide association study in primary sclerosing cholangitis detects multiple novel risk loci. Journal of hepatology. 2012 Aug;57(2):366-75. PubMed PMID: 22521342. Pubmed Central PMCID: PMC3399030. Epub 2012/04/24. eng.

69. Melum E, Franke A, Schramm C, Weismuller TJ, Gotthardt DN, Offner FA, et al. Genome-wide association analysis in primary sclerosing cholangitis identifies two non-HLA susceptibility loci. Nat Genet. 2011 Jan;43(1):17-9. PubMed PMID: 21151127. Pubmed Central PMCID: PMC4354850. Epub 2010/12/15. eng.

70. Karlsen TH, Franke A, Melum E, Kaser A, Hov JR, Balschun T, et al. Genome-wide association analysis in primary sclerosing cholangitis. Gastroenterology. 2010 Mar;138(3):1102-11. PubMed PMID: 19944697. Epub 2009/12/01. eng.

71. Hoyer KK, Dooms H, Barron L, Abbas AK. Interleukin-2 in the development and control of inflammatory disease. Immunological reviews. 2008 Dec;226:19-28. PubMed PMID: 19161413. Epub 2009/01/24. eng.

72. Chen L, Flies DB. Molecular mechanisms of T cell co-stimulation and co-inhibition. Nature reviews Immunology. 2013 Apr;13(4):227-42. PubMed PMID: 23470321. Pubmed Central PMCID: PMC3786574. Epub 2013/03/09. eng.

73. Liaskou E, Jeffery LE, Trivedi PJ, Reynolds GM, Suresh S, Bruns T, et al. Loss of CD28 expression by liver-infiltrating T cells contributes to pathogenesis of primary sclerosing cholangitis. Gastroenterology. 2014 Jul;147(1):221-32 e7. PubMed PMID: 24726754. Pubmed Central PMCID: PMC4961260. Epub 2014/04/15. eng.

74. Maroni L, Hohenester SD, van de Graaf SFJ, Tolenaars D, van Lienden K, Verheij J, et al. Knockout of the primary sclerosing cholangitis-risk gene Fut2 causes liver disease in mice. Hepatology (Baltimore, Md). 2017 Aug;66(2):542-54. PubMed PMID: 28056490. Epub 2017/01/06. eng.

75. Rupp C, Friedrich K, Folseraas T, Wannhoff A, Bode KA, Weiss KH, et al. Fut2 genotype is a risk factor for dominant stenosis and biliary candida infections in primary sclerosing cholangitis. Aliment Pharmacol Ther. 2014 Apr;39(8):873-82. PubMed PMID: 24612312. Epub 2014/03/13. eng.

76. Alberts R, de Vries EMG, Goode EC, Jiang X, Sampaziotis F, Rombouts K, et al. Genetic association analysis identifies variants associated with disease progression in primary sclerosing cholangitis. Gut. 2017 Aug 4. PubMed PMID: 28779025. Pubmed Central PMCID: 5797498.

77. Folseraas T, Liaskou E, Anderson CA, Karlsen TH. Genetics in PSC: what do the "risk genes" teach us? Clin Rev Allergy Immunol. 2015 Jun;48(2-3):154-64. PubMed PMID: 24736995.

78. Heymann F, Tacke F. Immunology in the liver--from homeostasis to disease. Nat Rev Gastroenterol Hepatol. 2016 Feb;13(2):88-110. PubMed PMID: 26758786.

79. Doherty DG. Immunity, tolerance and autoimmunity in the liver: A comprehensive review. Journal of autoimmunity. 2016 Jan;66:60-75. PubMed PMID: 26358406.

80. Berglin L, Bergquist A, Johansson H, Glaumann H, Jorns C, Lunemann S, et al. In situ characterization of intrahepatic non-parenchymal cells in PSC reveals phenotypic patterns associated with disease severity. PLoS One. 2014;9(8):e105375. PubMed PMID: 25141347. Pubmed Central PMCID: PMC4139378. Epub 2014/08/21. eng.

81. Panasiuk A, Prokopowicz D, Zak J, Panasiuk B, Wysocka J. Lymphocyte subpopulations in peripheral blood in primary sclerosing cholangitis. Hepatogastroenterology. 2004 Sep-Oct;51(59):1289-91. PubMed PMID: 15362735. Epub 2004/09/15. eng.

82. Racanelli V, Rehermann B. The liver as an immunological organ. Hepatology (Baltimore, Md). 2006 Feb;43(2 Suppl 1):S54-62. PubMed PMID: 16447271. Epub 2006/02/01. eng.

83. Woof JM, Kerr MA. The function of immunoglobulin A in immunity. The Journal of pathology. 2006 Jan;208(2):270-82. PubMed PMID: 16362985. Epub 2005/12/20. eng.

84. Berglin L, Bjorkstrom NK, Bergquist A. Primary sclerosing cholangitis is associated with autoreactive IgA antibodies against biliary epithelial cells. Scand J Gastroenterol. 2013 Jun;48(6):719-28. PubMed PMID: 23713804. Epub 2013/05/30. eng.

85. Oo YH, Banz V, Kavanagh D, Liaskou E, Withers DR, Humphreys E, et al. CXCR3-dependent recruitment and CCR6-mediated positioning of Th-17 cells in the inflamed liver. Journal of hepatology. 2012 Nov;57(5):1044-51. PubMed PMID: 22796894. Pubmed Central PMCID: PMC3994510. Epub 2012/07/17. eng.

86. Godfrey DI, Uldrich AP, McCluskey J, Rossjohn J, Moody DB. The burgeoning family of unconventional T cells. Nature immunology. 2015 Nov;16(11):1114-23. PubMed PMID: 26482978. Epub 2015/10/21. eng.

87. Kurioka A, Walker LJ, Klenerman P, Willberg CB. MAIT cells: new guardians of the liver. Clinical & translational immunology. 2016 Aug;5(8):e98. PubMed PMID: 27588203. Pubmed Central PMCID: 5007630.

88. Kjer-Nielsen L, Patel O, Corbett AJ, Le Nours J, Meehan B, Liu L, et al. MR1 presents microbial vitamin B metabolites to MAIT cells. Nature. 2012 Nov 29;491(7426):717-23. PubMed PMID: 23051753. Epub 2012/10/12. eng.

89. Le Bourhis L, Dusseaux M, Bohineust A, Bessoles S, Martin E, Premel V, et al. MAIT cells detect and efficiently lyse bacterially-infected epithelial cells. PLoS pathogens. 2013;9(10):e1003681. PubMed PMID: 24130485. Pubmed Central PMCID: PMC3795036. Epub 2013/10/17. eng.

90. Le Bourhis L, Martin E, Peguillet I, Guihot A, Froux N, Core M, et al. Antimicrobial activity of mucosal-associated invariant T cells. Nature immunology. 2010 Aug;11(8):701-8. PubMed PMID: 20581831. Epub 2010/06/29. eng.

91. Gold MC, Cerri S, Smyk-Pearson S, Cansler ME, Vogt TM, Delepine J, et al. Human mucosal associated invariant T cells detect bacterially infected cells. PLoS biology. 2010 Jun 29;8(6):e1000407. PubMed PMID: 20613858. Pubmed Central PMCID: PMC2893946. Epub 2010/07/09. eng.

92. Jeffery HC, van Wilgenburg B, Kurioka A, Parekh K, Stirling K, Roberts S, et al. Biliary epithelium and liver B cells exposed to bacteria activate intrahepatic MAIT cells through MR1. Journal of hepatology. 2016 May;64(5):1118-27. PubMed PMID: 26743076. Pubmed Central PMCID: PMC4822535. Epub 2016/01/09. eng.

93. Jo J, Tan AT, Ussher JE, Sandalova E, Tang XZ, Tan-Garcia A, et al. Toll-like receptor 8 agonist and bacteria trigger potent activation of innate immune cells in human liver. PLoS pathogens. 2014 Jun;10(6):e1004210. PubMed PMID: 24967632. Pubmed Central PMCID: PMC4072808. Epub 2014/06/27. eng.

94. Jenne CN, Kubes P. Immune surveillance by the liver. Nature immunology. 2013 Oct;14(10):996-1006. PubMed PMID: 24048121.

95. Allen K, Jaeschke H, Copple BL. Bile acids induce inflammatory genes in hepatocytes: a novel mechanism of inflammation during obstructive cholestasis. The American journal of pathology. 2011 Jan;178(1):175-86. PubMed PMID: 21224055. Pubmed Central PMCID: PMC3070591. Epub 2011/01/13. eng.

96. Mescher AL. Organs Associated with the Digestive Tract. Junqueira's Basic Histology, 14e. New York, NY: McGraw-Hill Education; 2016.

97. Marzioni M, Fava G, Alvaro D, Alpini G, Benedetti A. Control of cholangiocyte adaptive responses by visceral hormones and neuropeptides. Clin Rev Allergy Immunol. 2009 Feb;36(1):13-22. PubMed PMID: 18548352. Pubmed Central PMCID: 2628969.

98. Reynoso-Paz S, Coppel RL, Mackay IR, Bass NM, Ansari AA, Gershwin ME. The immunobiology of bile and biliary epithelium. Hepatology (Baltimore, Md). 1999 Aug;30(2):351-7. PubMed PMID: 10421640. Epub 1999/07/27. eng.

99. Schrumpf E, Tan C, Karlsen TH, Sponheim J, Bjorkstrom NK, Sundnes O, et al. The biliary epithelium presents antigens to and activates natural killer T cells. Hepatology (Baltimore, Md). 2015 Oct;62(4):1249-59. PubMed PMID: 25855031. Pubmed Central PMCID: PMC4589438. Epub 2015/04/10. eng.

100. Syal G, Fausther M, Dranoff JA. Advances in cholangiocyte immunobiology. Am J Physiol Gastrointest Liver Physiol. 2012 Nov 15;303(10):G1077-86. PubMed PMID: 22961800. Pubmed Central PMCID: PMC3517647. Epub 2012/09/11. eng.

101. Eksteen B, Miles A, Curbishley SM, Tselepis C, Grant AJ, Walker LSK, et al. Epithelial Inflammation Is Associated with CCL28 Production and the Recruitment of Regulatory T Cells Expressing CCR10. The Journal of Immunology. 2006;177(1):593-603.

102. Yokomuro S, Lunz JG, 3rd, Sakamoto T, Ezure T, Murase N, Demetris AJ. The effect of interleukin-6 (IL-6)/gp130 signalling on biliary epithelial cell growth, in vitro. Cytokine. 2000 Jun;12(6):727-30. PubMed PMID: 10843753. Epub 2000/06/14. eng.

103. Meng L, Quezada M, Levine P, Han Y, McDaniel K, Zhou T, et al. Functional role of cellular senescence in biliary injury. The American journal of pathology. 2015 Mar;185(3):602-9. PubMed PMID: 25619959. Pubmed Central PMCID: PMC4348464. Epub 2015/01/27. eng.

104. Chung BK, Karlsen TH, Folseraas T. Cholangiocytes in the pathogenesis of primary sclerosing cholangitis and development of cholangiocarcinoma. Biochimica et biophysica acta. 2017 Aug 25. PubMed PMID: 28844951.

105. Hohenester S, Wenniger LM, Paulusma CC, van Vliet SJ, Jefferson DM, Elferink RP, et al. A biliary HCO3- umbrella constitutes a protective mechanism against bile acid-induced injury in human cholangiocytes. Hepatology (Baltimore, Md). 2012 Jan;55(1):173-83. PubMed PMID: 21932391.

106. Keitel V, Ullmer C, Haussinger D. The membrane-bound bile acid receptor TGR5 (Gpbar-1) is localized in the primary cilium of cholangiocytes. Biological chemistry. 2010 Jul;391(7):785-9. PubMed PMID: 20623999. Epub 2010/07/14. eng.

107. Maillette de Buy Wenniger LJ, Hohenester S, Maroni L, van Vliet SJ, Oude Elferink RP, Beuers U. The Cholangiocyte Glycocalyx Stabilizes the 'Biliary HCO3 Umbrella': An Integrated Line of Defense against Toxic Bile Acids. Digestive diseases (Basel, Switzerland). 2015;33(3):397-407. PubMed PMID: 26045275. Epub 2015/06/06. eng.

108. O'Mahony CA, Vierling JM. Etiopathogenesis of primary sclerosing cholangitis. Seminars in liver disease. 2006 Feb;26(1):3-21. PubMed PMID: 16496229. Epub 2006/02/24. eng.

109. Tabibian JH, O'Hara SP, Lindor KD. Primary sclerosing cholangitis and the microbiota: current knowledge and perspectives on etiopathogenesis and emerging therapies. Scand J Gastroenterol. 2014 Aug;49(8):901-8. PubMed PMID: 24990660. Pubmed Central PMCID: PMC4210190. Epub 2014/07/06. eng.

110. Pollheimer MJ, Trauner M, Fickert P. Will we ever model PSC? - "it's hard to be a PSC model!". Clinics and research in hepatology and gastroenterology. 2011 Dec;35(12):792-804. PubMed PMID: 21703962. Epub 2011/06/28. eng.

111. Mueller T, Beutler C, Pico AH, Shibolet O, Pratt DS, Pascher A, et al. Enhanced innate immune responsiveness and intolerance to intestinal endotoxins in human biliary epithelial cells contributes to chronic cholangitis. Liver international : official journal of the International Association for the Study of the Liver. 2011 Nov;31(10):1574-88. PubMed PMID: 22093333. Epub 2011/11/19. eng.

112. Pohl J, Ring A, Stremmel W, Stiehl A. The role of dominant stenoses in bacterial infections of bile ducts in primary sclerosing cholangitis. Eur J Gastroenterol Hepatol. 2006 Jan;18(1):69-74. PubMed PMID: 16357622. Epub 2005/12/17. eng.

113. Tabibian JH, O'Hara SP, Splinter PL, Trussoni CE, LaRusso NF. Cholangiocyte senescence by way of N-ras activation is a characteristic of primary sclerosing

cholangitis. Hepatology (Baltimore, Md). 2014 Jun;59(6):2263-75. PubMed PMID: 24390753. Pubmed Central PMCID: PMC4167827. Epub 2014/01/07. eng.

114. Saarinen S, Olerup O, Broome U. Increased frequency of autoimmune diseases in patients with primary sclerosing cholangitis. Am J Gastroenterol. 2000 Nov;95(11):3195-9. PubMed PMID: 11095341. Epub 2000/11/30. eng.

115. Terjung B, Sohne J, Lechtenberg B, Gottwein J, Muennich M, Herzog V, et al. p-ANCAs in autoimmune liver disorders recognise human beta-tubulin isotype 5 and cross-react with microbial protein FtsZ. Gut. 2010 Jun;59(6):808-16. PubMed PMID: 19951907. Epub 2009/12/03. eng.

116. Eksteen B, Grant AJ, Miles A, Curbishley SM, Lalor PF, Hubscher SG, et al. Hepatic endothelial CCL25 mediates the recruitment of CCR9+ gut-homing lymphocytes to the liver in primary sclerosing cholangitis. The Journal of experimental medicine. 2004 Dec 6;200(11):1511-7. PubMed PMID: 15557349. Pubmed Central PMCID: 2211943.

117. Eksteen B, Mora JR, Haughton EL, Henderson NC, Lee-Turner L, Villablanca EJ, et al. Gut homing receptors on CD8 T cells are retinoic acid dependent and not maintained by liver dendritic or stellate cells. Gastroenterology. 2009 Jul;137(1):320-9. PubMed PMID: 19233184. Pubmed Central PMCID: PMC3201985. Epub 2009/02/24. eng.

118. Grant AJ, Lalor PF, Hubscher SG, Briskin M, Adams DH. MAdCAM-1 expressed in chronic inflammatory liver disease supports mucosal lymphocyte adhesion to hepatic endothelium (MAdCAM-1 in chronic inflammatory liver disease). Hepatology (Baltimore, Md). 2001 May;33(5):1065-72. PubMed PMID: 11343233. Epub 2001/05/09. eng.

119. Hillan KJ, Hagler KE, MacSween RN, Ryan AM, Renz ME, Chiu HH, et al. Expression of the mucosal vascular addressin, MAdCAM-1, in inflammatory liver disease. Liver. 1999 Dec;19(6):509-18. PubMed PMID: 10661685. Epub 2000/02/08. eng.

120. Farrant JM, Hayllar KM, Wilkinson ML, Karani J, Portmann BC, Westaby D, et al. Natural history and prognostic variables in primary sclerosing cholangitis. Gastroenterology. 1991 Jun;100(6):1710-7. PubMed PMID: 1850376. Epub 1991/06/01. eng.

121. Boberg KM, Bergquist A, Mitchell S, Pares A, Rosina F, Broome U, et al. Cholangiocarcinoma in primary sclerosing cholangitis: risk factors and clinical presentation. Scand J Gastroenterol. 2002 Oct;37(10):1205-11. PubMed PMID: 12408527. Epub 2002/11/01. eng.

122. Ponsioen CY, Vrouenraets SM, Prawirodirdjo W, Rajaram R, Rauws EA, Mulder CJ, et al. Natural history of primary sclerosing cholangitis and prognostic value of cholangiography in a Dutch population. Gut. 2002 Oct;51(4):562-6. PubMed PMID: 12235081. Pubmed Central PMCID: 1773389. Epub 2002/09/18. eng.

123. Takakura WR, Tabibian JH, Bowlus CL. The evolution of natural history of primary sclerosing cholangitis. Curr Opin Gastroenterol. 2017 Mar;33(2):71-7. PubMed PMID: 28030370. Pubmed Central PMCID: 5538137. Epub 2016/12/29. eng.

124. Bergquist A, Broome U. Hepatobiliary and extra-hepatic malignancies in primary sclerosing cholangitis. Best Pract Res Clin Gastroenterol. 2001 Aug;15(4):643-56. PubMed PMID: 11492973. Epub 2001/08/09. eng.

125. Harnois DM, Gores GJ, Ludwig J, Steers JL, LaRusso NF, Wiesner RH. Are patients with cirrhotic stage primary sclerosing cholangitis at risk for the development of

hepatocellular cancer? Journal of hepatology. 1997 Sep;27(3):512-6. PubMed PMID: 9314129. Epub 1997/10/06. eng.

126. Said K, Glaumann H, Bergquist A. Gallbladder disease in patients with primary sclerosing cholangitis. Journal of hepatology. 2008 Apr;48(4):598-605. PubMed PMID: 18222013. Epub 2008/01/29. eng.

127. Martin P, DiMartini A, Feng S, Brown R, Jr., Fallon M. Evaluation for liver transplantation in adults: 2013 practice guideline by the American Association for the Study of Liver Diseases and the American Society of Transplantation. Hepatology (Baltimore, Md). 2014 Mar;59(3):1144-65. PubMed PMID: 24716201. Epub 2014/04/10. eng.

128. Shetty K, Rybicki L, Carey WD. The Child-Pugh classification as a prognostic indicator for survival in primary sclerosing cholangitis. Hepatology (Baltimore, Md). 1997 May;25(5):1049-53. PubMed PMID: 9141416. Epub 1997/05/01. eng.

129. Kim WR, Therneau TM, Wiesner RH, Poterucha JJ, Benson JT, Malinchoc M, et al. A revised natural history model for primary sclerosing cholangitis. Mayo Clin Proc. 2000 Jul;75(7):688-94. PubMed PMID: 10907383. eng.

130. Petrovic BD, Nikolaidis P, Hammond NA, Martin JA, Petrovic PV, Desai PM, et al. Correlation between findings on MRCP and gadolinium-enhanced MR of the liver and a survival model for primary sclerosing cholangitis. Dig Dis Sci. 2007 Dec;52(12):3499-506. PubMed PMID: 17410447. Epub 2007/04/06. eng.

131. Support P. Clinical need in PSC and clinically meaningful change: What is important to patients? 2016. Available from: http://www.pscsupport.org.uk/sites/default/files/files/PSC Support Patient Survey Results(2).pdf.

132. Benito de Valle M, Rahman M, Lindkvist B, Bjornsson E, Chapman R, Kalaitzakis E. Factors that reduce health-related quality of life in patients with primary sclerosing cholangitis. Clin Gastroenterol Hepatol. 2012 Jul;10(7):769-75 e2. PubMed PMID: 22343690. Epub 2012/02/22. eng.

133. Gotthardt DN, Rupp C, Bruhin M, Schellberg D, Weiss KH, Stefan R, et al. Pruritus is associated with severely impaired quality of life in patients with primary sclerosing cholangitis. Eur J Gastroenterol Hepatol. 2014 Dec;26(12):1374-9. PubMed PMID: 25341059. Epub 2014/10/24. eng.

134. Raszeja-Wyszomirska J, Wunsch E, Krawczyk M, Rigopoulou EI, Bogdanos D, Milkiewicz P. Prospective evaluation of PBC-specific health-related quality of life questionnaires in patients with primary sclerosing cholangitis. Liver international : official journal of the International Association for the Study of the Liver. 2015 Jun;35(6):1764-71. PubMed PMID: 25388280. Epub 2014/11/13. eng.

135. Younossi ZM, Kiwi ML, Boparai N, Price LL, Guyatt G. Cholestatic liver diseases and health-related quality of life. Am J Gastroenterol. 2000 Feb;95(2):497-502. PubMed PMID: 10685757. Epub 2000/02/24. eng.

136. Zakharia K, Tabibian A, Lindor KD, Tabibian JH. Complications, symptoms, quality of life and pregnancy in cholestatic liver disease. Liver international : official journal of the International Association for the Study of the Liver. 2018 Mar;38(3):399-411. PubMed PMID: 28921801. Epub 2017/09/19. eng.

137. Cheung AC, Patel H, Meza-Cardona J, Cino M, Sockalingam S, Hirschfield GM. Factors that Influence Health-Related Quality of Life in Patients with Primary

Sclerosing Cholangitis. Dig Dis Sci. 2016 Jun;61(6):1692-9. PubMed PMID: 26743764. Epub 2016/01/09. eng.

138. Andersen IM, Fosby B, Boberg KM, Clausen OP, Jebsen P, Melum E, et al. Indications and Outcomes in Liver Transplantation in Patients With Primary Sclerosing Cholangitis in Norway. Transplantation direct. 2015 Oct;1(9):e39. PubMed PMID: 27500239. Pubmed Central PMCID: PMC4946487. Epub 2016/08/09. eng.

139. McCune WS, Shorb PE, Moscovitz H. Endoscopic cannulation of the ampulla of vater: a preliminary report. Ann Surg. 1968 May;167(5):752-6. PubMed PMID: 5646296. Pubmed Central PMCID: PMC1387128. Epub 1968/05/01. eng.

140. Chen LY, Goldberg HI. Sclerosing cholangitis: broad spectrum of radiographic features. Gastrointestinal radiology. 1984;9(1):39-47. PubMed PMID: 6724238. Epub 1984/01/01. eng.

141. Majoie CB, Reeders JW, Sanders JB, Huibregtse K, Jansen PL. Primary sclerosing cholangitis: a modified classification of cholangiographic findings. AJR Am J Roentgenol. 1991 Sep;157(3):495-7. PubMed PMID: 1651643. Epub 1991/09/01. eng.

142. Ponsioen CY, Reitsma JB, Boberg KM, Aabakken L, Rauws EA, Schrumpf E. Validation of a cholangiographic prognostic model in primary sclerosing cholangitis. Endoscopy. 2010 Sep;42(9):742-7. PubMed PMID: 20623444. Epub 2010/07/14. eng.

143. Rossi G, Sciveres M, Maruzzelli L, Curcio G, Riva S, Traina M, et al. Diagnosis of sclerosing cholangitis in children: blinded, comparative study of magnetic resonance versus endoscopic cholangiography. Clinics and research in hepatology and gastroenterology. 2013 Dec;37(6):596-601. PubMed PMID: 23830985. Epub 2013/07/09. eng.

144. Moff SL, Kamel IR, Eustace J, Lawler LP, Kantsevoy S, Kalloo AN, et al. Diagnosis of primary sclerosing cholangitis: a blinded comparative study using magnetic resonance cholangiography and endoscopic retrograde cholangiography. Gastrointestinal endoscopy. 2006 Aug;64(2):219-23. PubMed PMID: 16860072. Epub 2006/07/25. eng.

145. Lindor KD, Kowdley KV, Harrison ME. ACG Clinical Guideline: Primary Sclerosing Cholangitis. Am J Gastroenterol. 2015 May;110(5):646-59; quiz 60. PubMed PMID: 25869391. Epub 2015/04/15. eng.

146. Committee ASoP, Chathadi KV, Chandrasekhara V, Acosta RD, Decker GA, Early DS, et al. The role of ERCP in benign diseases of the biliary tract. Gastrointestinal endoscopy. 2015 Apr;81(4):795-803. PubMed PMID: 25665931.

147. Dumonceau JM, Tringali A, Blero D, Deviere J, Laugiers R, Heresbach D, et al. Biliary stenting: indications, choice of stents and results: European Society of Gastrointestinal Endoscopy (ESGE) clinical guideline. Endoscopy. 2012 Mar;44(3):277-98. PubMed PMID: 22297801.

148. Hammel P, Couvelard A, O'Toole D, Ratouis A, Sauvanet A, Flejou JF, et al. Regression of liver fibrosis after biliary drainage in patients with chronic pancreatitis and stenosis of the common bile duct. N Engl J Med. 2001 Feb 8;344(6):418-23. PubMed PMID: 11172178. Epub 2001/02/15. eng.

149. Stiehl A, Rudolph G, Kloters-Plachky P, Sauer P, Walker S. Development of dominant bile duct stenoses in patients with primary sclerosing cholangitis treated with ursodeoxycholic acid: outcome after endoscopic treatment. Journal of hepatology. 2002 Feb;36(2):151-6. PubMed PMID: 11830325. Epub 2002/02/07. eng.

150. Ruiz A, Lemoinne S, Carrat F, Corpechot C, Chazouilleres O, Arrive L. Radiologic course of primary sclerosing cholangitis: assessment by three-dimensional magnetic resonance cholangiography and predictive features of progression. Hepatology (Baltimore, Md). 2014 Jan;59(1):242-50. PubMed PMID: 23857427. Epub 2013/07/17. eng.

151. Bjornsson E, Lindqvist-Ottosson J, Asztely M, Olsson R. Dominant strictures in patients with primary sclerosing cholangitis. Am J Gastroenterol. 2004 Mar;99(3):502-8. PubMed PMID: 15056092. Epub 2004/04/02. eng.

152. Gotthardt DN, Rudolph G, Kloters-Plachky P, Kulaksiz H, Stiehl A. Endoscopic dilation of dominant stenoses in primary sclerosing cholangitis: outcome after long-term treatment. Gastrointestinal endoscopy. 2010 Mar;71(3):527-34. PubMed PMID: 20189511. Epub 2010/03/02. eng.

153. Rudolph G, Gotthardt D, Kloters-Plachky P, Kulaksiz H, Rost D, Stiehl A. Influence of dominant bile duct stenoses and biliary infections on outcome in primary sclerosing cholangitis. Journal of hepatology. 2009 Jul;51(1):149-55. PubMed PMID: 19410324. Epub 2009/05/05. eng.

154. Gluck M, Cantone NR, Brandabur JJ, Patterson DJ, Bredfeldt JE, Kozarek RA. A twenty-year experience with endoscopic therapy for symptomatic primary sclerosing cholangitis. J Clin Gastroenterol. 2008 Oct;42(9):1032-9. PubMed PMID: 18580600. Epub 2008/06/27. eng.

155. Kaya M, Petersen BT, Angulo P, Baron TH, Andrews JC, Gostout CJ, et al. Balloon dilation compared to stenting of dominant strictures in primary sclerosing cholangitis. Am J Gastroenterol. 2001 Apr;96(4):1059-66. PubMed PMID: 11316147. Epub 2001/04/24. eng.

156. Baluyut AR, Sherman S, Lehman GA, Hoen H, Chalasani N. Impact of endoscopic therapy on the survival of patients with primary sclerosing. Gastrointestinal endoscopy. 2001 Mar;53(3):308-12. PubMed PMID: 11231388. Epub 2001/03/07. eng.

157. Ponsioen CY, Lam K, van Milligen de Wit AW, Huibregtse K, Tytgat GN. Four years experience with short term stenting in primary sclerosing cholangitis. Am J Gastroenterol. 1999 Sep;94(9):2403-7. PubMed PMID: 10483999. Epub 1999/09/14. eng.

158. van Milligen de Wit AW, van Bracht J, Rauws EA, Jones EA, Tytgat GN, Huibregtse K. Endoscopic stent therapy for dominant extrahepatic bile duct strictures in primary sclerosing cholangitis. Gastrointestinal endoscopy. 1996 Sep;44(3):293-9. PubMed PMID: 8885349. Epub 1996/09/01. eng.

159. Ponsioen C, Arnelo U, Bergquist A, Rauws E, Paulsen V, Cantu P, et al. Multicenter randomized trial comparing short-term stenting versus balloon dilatation for dominant strictures in primary sclerosing cholangitis. Journal of hepatology.66(1):S1-S2.

160. Cotton PB, Lehman G, Vennes J, Geenen JE, Russell RC, Meyers WC, et al. Endoscopic sphincterotomy complications and their management: an attempt at consensus. Gastrointestinal endoscopy. 1991 May-Jun;37(3):383-93. PubMed PMID: 2070995. Epub 1991/05/01. eng.

161. Andriulli A, Loperfido S, Napolitano G, Niro G, Valvano MR, Spirito F, et al. Incidence rates of post-ERCP complications: a systematic survey of prospective studies. Am J Gastroenterol. 2007 Aug;102(8):1781-8. PubMed PMID: 17509029. Epub 2007/05/19. eng.

162. Cotton PB, Garrow DA, Gallagher J, Romagnuolo J. Risk factors for complications after ERCP: a multivariate analysis of 11,497 procedures over 12 years.

Gastrointestinal endoscopy. 2009 Jul;70(1):80-8. PubMed PMID: 19286178. Epub 2009/03/17. eng.

163. Jeurnink SM, Siersema PD, Steyerberg EW, Dees J, Poley JW, Haringsma J, et al. Predictors of complications after endoscopic retrograde cholangiopancreatography: a prognostic model for early discharge. Surg Endosc. 2011 Sep;25(9):2892-900. PubMed PMID: 21455806. Pubmed Central PMCID: 3160559. Epub 2011/04/02. eng.

164. Vandervoort J, Soetikno RM, Tham TC, Wong RC, Ferrari AP, Montes H, et al. Risk factors for complications after performance of ERCP. Gastrointestinal endoscopy. 2002 Nov;56(5):652-6. PubMed PMID: 12397271. eng.

165. Williams EJ, Taylor S, Fairclough P, Hamlyn A, Logan RF, Martin D, et al. Risk factors for complication following ERCP; results of a large-scale, prospective multicenter study. Endoscopy. 2007 Sep;39(9):793-801. PubMed PMID: 17703388.

166. Bangarulingam SY, Gossard AA, Petersen BT, Ott BJ, Lindor KD. Complications of endoscopic retrograde cholangiopancreatography in primary sclerosing cholangitis. Am J Gastroenterol. 2009 Apr;104(4):855-60. PubMed PMID: 19259076. Epub 2009/03/05. eng.

167. Etzel JP, Eng SC, Ko CW, Lee SD, Saunders MD, Tung BY, et al. Complications after ERCP in patients with primary sclerosing cholangitis. Gastrointestinal endoscopy. 2008 Apr;67(4):643-8. PubMed PMID: 18061595. Epub 2007/12/07. eng.

168. Ismail S, Kylanpaa L, Mustonen H, Halttunen J, Lindstrom O, Jokelainen K, et al. Risk factors for complications of ERCP in primary sclerosing cholangitis. Endoscopy. 2012 Dec;44(12):1133-8. PubMed PMID: 23108808.

169. Lee JG, Schutz SM, England RE, Leung JW, Cotton PB. Endoscopic therapy of sclerosing cholangitis. Hepatology (Baltimore, Md). 1995 Mar;21(3):661-7. PubMed PMID: 7875664. eng.

170. van den Hazel SJ, Wolfhagen EH, van Buuren HR, van de Meeberg PC, Van Leeuwen DJ. Prospective risk assessment of endoscopic retrograde cholangiography in patients with primary sclerosing cholangitis. Dutch PSC Study Group. Endoscopy. 2000 Oct;32(10):779-82. PubMed PMID: 11068837. eng.

171. Razumilava N, Gores GJ. Cholangiocarcinoma. The Lancet. 2014 //;383(9935):2168-79.

172. Bergquist A, von Seth E. Epidemiology of cholangiocarcinoma. Best Pract Res Clin Gastroenterol. 2015 Apr;29(2):221-32. PubMed PMID: 25966423. Epub 2015/05/13. eng.

173. Jepsen P, Vilstrup H, Tarone RE, Friis S, Sorensen HT. Incidence rates of intra- and extrahepatic cholangiocarcinomas in Denmark from 1978 through 2002. Journal of the National Cancer Institute. 2007 Jun 6;99(11):895-7. PubMed PMID: 17551150. Epub 2007/06/07. eng.

174. Khan SA, Davidson BR, Goldin RD, Heaton N, Karani J, Pereira SP, et al. Guidelines for the diagnosis and treatment of cholangiocarcinoma: an update. Gut. 2012 Dec;61(12):1657-69. PubMed PMID: 22895392. Epub 2012/08/17. eng.

175. Bergquist A, Ekbom A, Olsson R, Kornfeldt D, Loof L, Danielsson A, et al. Hepatic and extrahepatic malignancies in primary sclerosing cholangitis. Journal of hepatology. 2002 Mar;36(3):321-7. PubMed PMID: 11867174. Epub 2002/02/28. eng.

176. Burak K, Angulo P, Pasha TM, Egan K, Petz J, Lindor KD. Incidence and risk factors for cholangiocarcinoma in primary sclerosing cholangitis. Am J Gastroenterol. 2004 Mar;99(3):523-6. PubMed PMID: 15056096. Epub 2004/04/02. eng.

177. Fevery J, Verslype C, Lai G, Aerts R, Van Steenbergen W. Incidence, diagnosis, and therapy of cholangiocarcinoma in patients with primary sclerosing cholangitis. Dig Dis Sci. 2007 Nov;52(11):3123-35. PubMed PMID: 17431781. Epub 2007/04/14. eng.

178. Morris-Stiff G, Bhati C, Olliff S, Hubscher S, Gunson B, Mayer D, et al. Cholangiocarcinoma complicating primary sclerosing cholangitis: a 24-year experience. Dig Surg. 2008;25(2):126-32. PubMed PMID: 18446034. Epub 2008/05/01. eng.

179. Folseraas T, Boberg KM. Cancer Risk and Surveillance in Primary Sclerosing Cholangitis. Clin Liver Dis. 2016 Feb;20(1):79-98. PubMed PMID: 26593292. Epub 2015/11/26. eng.

180. Friman S, Foss A, Isoniemi H, Olausson M, Hockerstedt K, Yamamoto S, et al. Liver transplantation for cholangiocarcinoma: selection is essential for acceptable results. Scand J Gastroenterol. 2011 Mar;46(3):370-5. PubMed PMID: 21073376.

181. Rosen CB, Heimbach JK, Gores GJ. Liver transplantation for cholangiocarcinoma. Transpl Int. 2010 Jul;23(7):692-7. PubMed PMID: 20497401. Epub 2010/05/26. eng.

182. Friman S. Cholangiocarcinoma--current treatment options. Scandinavian journal of surgery : SJS : official organ for the Finnish Surgical Society and the Scandinavian Surgical Society. 2011;100(1):30-4. PubMed PMID: 21491796. Epub 2011/04/16. eng.

183. Ahrendt SA, Pitt HA, Nakeeb A, Klein AS, Lillemoe KD, Kalloo AN, et al. Diagnosis and management of cholangiocarcinoma in primary sclerosing cholangitis. J Gastrointest Surg. 1999 Jul-Aug;3(4):357-67; discussion 67-8. PubMed PMID: 10482687. Epub 1999/09/11. eng.

184. Chalasani N, Baluyut A, Ismail A, Zaman A, Sood G, Ghalib R, et al. Cholangiocarcinoma in patients with primary sclerosing cholangitis: a multicenter case-control study. Hepatology (Baltimore, Md). 2000 Jan;31(1):7-11. PubMed PMID: 10613720. Epub 1999/12/29. eng.

185. Melum E, Karlsen TH, Schrumpf E, Bergquist A, Thorsby E, Boberg KM, et al. Cholangiocarcinoma in primary sclerosing cholangitis is associated with NKG2D polymorphisms. Hepatology (Baltimore, Md). 2008 Jan;47(1):90-6. PubMed PMID: 18023027. Epub 2007/11/21. eng.

186. Lewis JT, Talwalkar JA, Rosen CB, Smyrk TC, Abraham SC. Precancerous bile duct pathology in end-stage primary sclerosing cholangitis, with and without cholangiocarcinoma. Am J Surg Pathol. 2010 Jan;34(1):27-34. PubMed PMID: 19898228. Epub 2009/11/10. eng.

187. Kerr SE, Barr Fritcher EG, Campion MB, Voss JS, Kipp BR, Halling KC, et al. Biliary dysplasia in primary sclerosing cholangitis harbors cytogenetic abnormalities similar to cholangiocarcinoma. Hum Pathol. 2014 Sep;45(9):1797-804. PubMed PMID: 25027853.

188. Charatcharoenwitthaya P, Enders FB, Halling KC, Lindor KD. Utility of serum tumor markers, imaging, and biliary cytology for detecting cholangiocarcinoma in primary sclerosing cholangitis. Hepatology (Baltimore, Md). 2008 Oct;48(4):1106-17. PubMed PMID: 18785620. eng.

189. Sinakos E, Saenger AK, Keach J, Kim WR, Lindor KD. Many patients with primary sclerosing cholangitis and increased serum levels of carbohydrate antigen 19-9 do not have cholangiocarcinoma. Clin Gastroenterol Hepatol. 2011 May;9(5):434-9 e1. PubMed PMID: 21334457. Epub 2011/02/22. eng.

190. Eaton JE, Gossard AA, Talwalkar JA. Recall processes for biliary cytology in primary sclerosing cholangitis. Curr Opin Gastroenterol. 2014 May;30(3):287-94. PubMed PMID: 24686433.

191. Boberg KM, Jebsen P, Clausen OP, Foss A, Aabakken L, Schrumpf E. Diagnostic benefit of biliary brush cytology in cholangiocarcinoma in primary sclerosing cholangitis. Journal of hepatology. 2006 Oct;45(4):568-74. PubMed PMID: 16879890. Epub 2006/08/02. eng.

192. Trikudanathan G, Navaneethan U, Njei B, Vargo JJ, Parsi MA. Diagnostic yield of bile duct brushings for cholangiocarcinoma in primary sclerosing cholangitis: a systematic review and meta-analysis. Gastrointestinal endoscopy. 2014 May;79(5):783-9. PubMed PMID: 24140129.

193. Harewood GC, Baron TH, Stadheim LM, Kipp BR, Sebo TJ, Salomao DR. Prospective, blinded assessment of factors influencing the accuracy of biliary cytology interpretation. Am J Gastroenterol. 2004 Aug;99(8):1464-9. PubMed PMID: 15307861. Epub 2004/08/17. eng.

194. Adamsen S, Olsen M, Jendresen MB, Holck S, Glenthoj A. Endobiliary brush biopsy: Intra- and interobserver variation in cytological evaluation of brushings from bile duct strictures. Scand J Gastroenterol. 2006 May;41(5):597-603. PubMed PMID: 16638704. Epub 2006/04/28. eng.

195. Kipp BR, Barr Fritcher EG, Pettengill JE, Halling KC, Clayton AC. Improving the accuracy of pancreatobiliary tract cytology with fluorescence in situ hybridization: a molecular test with proven clinical success. Cancer cytopathology. 2013 Nov;121(11):610-9. PubMed PMID: 23633236. Epub 2013/05/02. eng.

196. Barr Fritcher EG, Voss JS, Jenkins SM, Lingineni RK, Clayton AC, Roberts LR, et al. Primary sclerosing cholangitis with equivocal cytology: fluorescence in situ hybridization and serum CA 19-9 predict risk of malignancy. Cancer cytopathology. 2013 Dec;121(12):708-17. PubMed PMID: 23839915.

197. Bergquist A, Tribukait B, Glaumann H, Broome U. Can DNA cytometry be used for evaluation of malignancy and premalignancy in bile duct strictures in primary sclerosing cholangitis? Journal of hepatology. 2000 Dec;33(6):873-7. PubMed PMID: 11131447. Epub 2000/12/29. eng.

198. Moreno Luna LE, Kipp B, Halling KC, Sebo TJ, Kremers WK, Roberts LR, et al. Advanced cytologic techniques for the detection of malignant pancreatobiliary strictures. Gastroenterology. 2006 Oct;131(4):1064-72. PubMed PMID: 17030177. Pubmed Central PMCID: PMC1769444. Epub 2006/10/13. eng.

199. Bangarulingam SY, Bjornsson E, Enders F, Barr Fritcher EG, Gores G, Halling KC, et al. Long-term outcomes of positive fluorescence in situ hybridization tests in primary sclerosing cholangitis. Hepatology (Baltimore, Md). 2010 Jan;51(1):174-80. PubMed PMID: 19877179.

200. Navaneethan U, Njei B, Venkatesh PG, Vargo JJ, Parsi MA. Fluorescence in situ hybridization for diagnosis of cholangiocarcinoma in primary sclerosing cholangitis: a

systematic review and meta-analysis. Gastrointestinal endoscopy. 2014 Jun;79(6):943-50 e3. PubMed PMID: 24360654.

201. Barr Fritcher EG, Kipp BR, Voss JS, Clayton AC, Lindor KD, Halling KC, et al. Primary sclerosing cholangitis patients with serial polysomy fluorescence in situ hybridization results are at increased risk of cholangiocarcinoma. Am J Gastroenterol. 2011 Nov;106(11):2023-8. PubMed PMID: 21844920.

202. Eaton JE, Barr Fritcher EG, Gores GJ, Atkinson EJ, Tabibian JH, Topazian MD, et al. Biliary multifocal chromosomal polysomy and cholangiocarcinoma in primary sclerosing cholangitis. Am J Gastroenterol. 2015 Feb;110(2):299-309. PubMed PMID: 25623660. Pubmed Central PMCID: PMC4349337. Epub 2015/01/28. eng.

203. Tischendorf JJ, Kruger M, Trautwein C, Duckstein N, Schneider A, Manns MP, et al. Cholangioscopic characterization of dominant bile duct stenoses in patients with primary sclerosing cholangitis. Endoscopy. 2006 Jul;38(7):665-9. PubMed PMID: 16673310. eng.

204. Chen YK, Pleskow DK. SpyGlass single-operator peroral cholangiopancreatoscopy system for the diagnosis and therapy of bile-duct disorders: a clinical feasibility study (with video). Gastrointestinal endoscopy. 2007 May;65(6):832-41. PubMed PMID: 17466202. eng.

205. Draganov PV, Lin T, Chauhan S, Wagh MS, Hou W, Forsmark CE. Prospective evaluation of the clinical utility of ERCP-guided cholangiopancreatoscopy with a new direct visualization system. Gastrointestinal endoscopy. 2011 May;73(5):971-9. PubMed PMID: 21419408. eng.

206. Ramchandani M, Reddy DN, Gupta R, Lakhtakia S, Tandan M, Darisetty S, et al. Role of single-operator peroral cholangioscopy in the diagnosis of indeterminate biliary lesions: a single-center, prospective study. Gastrointestinal endoscopy. 2011 Sep;74(3):511-9. PubMed PMID: 21737076. eng.

207. Enochsson L, Swahn F, Arnelo U, Nilsson M, Lohr M, Persson G. Nationwide, population-based data from 11,074 ERCP procedures from the Swedish Registry for Gallstone Surgery and ERCP. Gastrointestinal endoscopy. 2010 Dec;72(6):1175-84, 84 e1-3. PubMed PMID: 20970787. Epub 2010/10/26. eng.

208. Christensen M, Matzen P, Schulze S, Rosenberg J. Complications of ERCP: a prospective study. Gastrointestinal endoscopy. 2004 Nov;60(5):721-31. PubMed PMID: 15557948. Epub 2004/11/24. eng.

209. Foutch PG. A prospective assessment of results for needle-knife papillotomy and standard endoscopic sphincterotomy. Gastrointestinal endoscopy. 1995 Jan;41(1):25-32. PubMed PMID: 7698621. Epub 1995/01/01. eng.

210. Lindberg B, Arnelo U, Bergquist A, Thorne A, Hjerpe A, Granqvist S, et al. Diagnosis of biliary strictures in conjunction with endoscopic retrograde cholangiopancreaticography, with special reference to patients with primary sclerosing cholangitis. Endoscopy. 2002 Nov;34(11):909-16. PubMed PMID: 12430077. eng.

211. Cotton PB, Eisen GM, Aabakken L, Baron TH, Hutter MM, Jacobson BC, et al. A lexicon for endoscopic adverse events: report of an ASGE workshop. Gastrointestinal endoscopy. 2010 Mar;71(3):446-54. PubMed PMID: 20189503. eng.

212. Magro F, Gionchetti P, Eliakim R, Ardizzone S, Armuzzi A, Barreiro-de Acosta M, et al. Third European Evidence-based Consensus on Diagnosis and Management

of Ulcerative Colitis. Part 1: Definitions, Diagnosis, Extra-intestinal Manifestations, Pregnancy, Cancer Surveillance, Surgery, and Ileo-anal Pouch Disorders. Journal of Crohn's and Colitis. 2017;11(6):649-70.

213. Dias J, Sobkowiak MJ, Sandberg JK, Leeansyah E. Human MAIT-cell responses to Escherichia coli: activation, cytokine production, proliferation, and cytotoxicity. Journal of leukocyte biology. 2016 Jul;100(1):233-40. PubMed PMID: 27034405. Pubmed Central PMCID: PMC4946616. Epub 2016/04/02. eng.

214. Hengst J, Theorell J, Deterding K, Potthoff A, Dettmer A, Ljunggren HG, et al. High-resolution determination of human immune cell signatures from fine-needle liver aspirates. European journal of immunology. 2015 Jul;45(7):2154-7. PubMed PMID: 25824697. Epub 2015/04/01. eng.

215. Martin E, Treiner E, Duban L, Guerri L, Laude H, Toly C, et al. Stepwise development of MAIT cells in mouse and human. PLoS biology. 2009 Mar 10;7(3):e54. PubMed PMID: 19278296. Pubmed Central PMCID: PMC2653554 co-owns the filled patent for the 3C10 antibody. EM has received salary through this funding. Epub 2009/03/13. eng.

216. Amir el AD, Davis KL, Tadmor MD, Simonds EF, Levine JH, Bendall SC, et al. viSNE enables visualization of high dimensional single-cell data and reveals phenotypic heterogeneity of leukemia. Nature biotechnology. 2013 Jun;31(6):545-52. PubMed PMID: 23685480. Pubmed Central PMCID: PMC4076922. Epub 2013/05/21. eng.

217. Tang XZ, Jo J, Tan AT, Sandalova E, Chia A, Tan KC, et al. IL-7 licenses activation of human liver intrasinusoidal mucosal-associated invariant T cells. J Immunol. 2013 Apr 1;190(7):3142-52. PubMed PMID: 23447689. Epub 2013/03/01. eng.

218. Alkhatib AA, Hilden K Fau - Adler DG, Adler DG. Comorbidities, sphincterotomy, and balloon dilation predict post-ERCP adverse events in PSC patients: operator experience is protective. (1573-2568 (Electronic)). eng.

219. Enns R, Eloubeidi Ma Fau - Mergener K, Mergener K Fau - Jowell PS, Jowell Ps Fau - Branch MS, Branch Ms Fau - Baillie J, Baillie J. Predictors of successful clinical and laboratory outcomes in patients with primary sclerosing cholangitis undergoing endoscopic retrograde cholangiopancreatography. (0835-7900 (Print)). eng.

220. Navaneethan U, Jegadeesan R, Nayak S, Lourdusamy V, Sanaka MR, Vargo JJ, et al. ERCP-related adverse events in patients with primary sclerosing cholangitis. Gastrointestinal endoscopy. 2014 Jul 30. PubMed PMID: 25085336. Epub 2014/08/03. Eng.

221. Lindstrom E, Bodemar G, Ryden BO, Ihse I. Pancreatic ductal morphology and exocrine function in primary sclerosing cholangitis. Acta chirurgica Scandinavica. 1990 Jun-Jul;156(6-7):451-6. PubMed PMID: 2368550.

222. Said K, Albiin N, Lindberg B, Brismar TB, Karrar A, Permert J, et al. Pancreatic duct changes are not associated with early signs of chronic pancreatitis at magnetic resonance imaging (MRI) in patients with primary sclerosing cholangitis. Scand J Gastroenterol. 2010 Aug;45(7-8):980-6. PubMed PMID: 20384530. eng.

223. Wu HM, Dixon E, May GR, Sutherland FR. Management of perforation after endoscopic retrograde cholangiopancreatography (ERCP): a population-based review. HPB : the official journal of the International Hepato Pancreato Biliary Association. 2006;8(5):393-9. PubMed PMID: 18333093. Pubmed Central PMCID: PMC2020744. Epub 2008/03/12. eng.

224. Dumonceau JM, Andriulli A, Elmunzer BJ, Mariani A, Meister T, Deviere J, et al. Prophylaxis of post-ERCP pancreatitis: European Society of Gastrointestinal Endoscopy (ESGE) Guideline - updated June 2014. Endoscopy. 2014 Sep;46(9):799-815. PubMed PMID: 25148137.

225. Draganov PV, Chauhan S, Wagh MS, Gupte AR, Lin T, Hou W, et al. Diagnostic accuracy of conventional and cholangioscopy-guided sampling of indeterminate biliary lesions at the time of ERCP: a prospective, long-term follow-up study. Gastrointestinal endoscopy. 2012 Feb;75(2):347-53. PubMed PMID: 22248602. Epub 2012/01/18. eng.

226. Siddiqui AA, Mehendiratta V, Jackson W, Loren DE, Kowalski TE, Eloubeidi MA. Identification of cholangiocarcinoma by using the Spyglass Spyscope system for peroral cholangioscopy and biopsy collection. Clin Gastroenterol Hepatol. 2012 May;10(5):466-71; quiz e48. PubMed PMID: 22178463.

227. Kalaitzakis E, Webster GJ, Oppong KW, Kallis Y, Vlavianos P, Huggett M, et al. Diagnostic and therapeutic utility of single-operator peroral cholangioscopy for indeterminate biliary lesions and bile duct stones. Eur J Gastroenterol Hepatol. 2012 Jun;24(6):656-64. PubMed PMID: 22433791. Epub 2012/03/22. eng.

228. Sethi A, Chen YK, Austin GL, Brown WR, Brauer BC, Fukami NN, et al. ERCP with cholangiopancreatoscopy may be associated with higher rates of complications than ERCP alone: a single-center experience. Gastrointestinal endoscopy. 2011 Feb;73(2):251-6. PubMed PMID: 21106195. Epub 2010/11/26. eng.

229. Awadallah NS, Chen YK, Piraka C, Antillon MR, Shah RJ. Is there a role for cholangioscopy in patients with primary sclerosing cholangitis? Am J Gastroenterol. 2006 Feb;101(2):284-91. PubMed PMID: 16454832. Epub 2006/02/04. eng.

230. Siiki A, Rinta-Kiikka I Fau - Koivisto T, Koivisto T Fau - Vasama K, Vasama K Fau - Sand J, Sand J Fau - Laukkarinen J, Laukkarinen J. Spyglass single-operator peroral cholangioscopy seems promising in the evaluation of primary sclerosing cholangitis-related biliary strictures. (1502-7708 (Electronic)). eng.

231. Azeem N, Gostout CJ, Knipschield M, Baron TH. Cholangioscopy with narrow-band imaging in patients with primary sclerosing cholangitis undergoing ERCP. Gastrointestinal endoscopy. 2014 May;79(5):773-9 e2. PubMed PMID: 24206748. Epub 2013/11/12. eng.

232. von Seth E, Arnelo U, Enochsson L, Bergquist A. Primary sclerosing cholangitis increases the risk for pancreatitis after endoscopic retrograde cholangiopancreatography. Liver international : official journal of the International Association for the Study of the Liver. 2015 Jan;35(1):254-62. PubMed PMID: 25041074. Epub 2014/07/22. Eng.

233. Quinn KP, Tabibian JH, Lindor KD. Clinical implications of serial versus isolated biliary fluorescence in situ hybridization (FISH) polysomy in primary sclerosing cholangitis. Scand J Gastroenterol. 2017 Apr;52(4):377-81. PubMed PMID: 27908204.

234. DeHaan RD, Kipp BR, Smyrk TC, Abraham SC, Roberts LR, Halling KC. An assessment of chromosomal alterations detected by fluorescence in situ hybridization and p16 expression in sporadic and primary sclerosing cholangitis-associated cholangiocarcinomas. Hum Pathol. 2007 Mar;38(3):491-9. PubMed PMID: 17239935. Epub 2007/01/24. eng.

235. Hengst J, Strunz B, Deterding K, Ljunggren HG, Leeansyah E, Manns MP, et al. Nonreversible MAIT cell-dysfunction in chronic hepatitis C virus infection despite

successful interferon-free therapy. European journal of immunology. 2016 Sep;46(9):2204-10. PubMed PMID: 27296288.

236. Riva A, Patel V, Kurioka A, Jeffery HC, Wright G, Tarff S, et al. Mucosa-associated invariant T cells link intestinal immunity with antibacterial immune defects in alcoholic liver disease. Gut. 2017 Nov 2. PubMed PMID: 29097439. Epub 2017/11/04. eng.

237. Miyazaki Y, Miyake S, Chiba A, Lantz O, Yamamura T. Mucosal-associated invariant T cells regulate Th1 response in multiple sclerosis. International immunology. 2011 Sep;23(9):529-35. PubMed PMID: 21712423. Epub 2011/06/30. eng.

238. Menon B, Gullick NJ, Walter GJ, Rajasekhar M, Garrood T, Evans HG, et al. Interleukin-17+CD8+ T cells are enriched in the joints of patients with psoriatic arthritis and correlate with disease activity and joint damage progression. Arthritis & rheumatology (Hoboken, NJ). 2014 May;66(5):1272-81. PubMed PMID: 24470327. Pubmed Central PMCID: PMC4158887. Epub 2014/01/29. eng.

239. Serriari NE, Eoche M, Lamotte L, Lion J, Fumery M, Marcelo P, et al. Innate mucosal-associated invariant T (MAIT) cells are activated in inflammatory bowel diseases. Clin Exp Immunol. 2014 May;176(2):266-74. PubMed PMID: 24450998. Pubmed Central PMCID: 3992039.

240. Cosgrove C, Ussher JE, Rauch A, Gartner K, Kurioka A, Huhn MH, et al. Early and nonreversible decrease of CD161++ /MAIT cells in HIV infection. Blood. 2013 Feb 7;121(6):951-61. PubMed PMID: 23255555. Pubmed Central PMCID: PMC3567342. Epub 2012/12/21. eng.

241. Leeansyah E, Ganesh A, Quigley MF, Sonnerborg A, Andersson J, Hunt PW, et al. Activation, exhaustion, and persistent decline of the antimicrobial MR1-restricted MAIT-cell population in chronic HIV-1 infection. Blood. 2013 Feb 14;121(7):1124-35. PubMed PMID: 23243281. Pubmed Central PMCID: PMC3575756. Epub 2012/12/18. eng.

242. Cho YN, Kee SJ, Kim TJ, Jin HM, Kim MJ, Jung HJ, et al. Mucosal-associated invariant T cell deficiency in systemic lupus erythematosus. J Immunol. 2014 Oct 15;193(8):3891-901. PubMed PMID: 25225673. Epub 2014/09/17. eng.

243. Magalhaes I, Pingris K, Poitou C, Bessoles S, Venteclef N, Kiaf B, et al. Mucosal-associated invariant T cell alterations in obese and type 2 diabetic patients. J Clin Invest. 2015 Apr;125(4):1752-62. PubMed PMID: 25751065. Pubmed Central PMCID: PMC4396481. Epub 2015/03/10. eng.

244. Sandler NG, Koh C, Roque A, Eccleston JL, Siegel RB, Demino M, et al. Host response to translocated microbial products predicts outcomes of patients with HBV or HCV infection. Gastroenterology. 2011 Oct;141(4):1220-30, 30 e1-3. PubMed PMID: 21726511. Pubmed Central PMCID: PMC3186837. Epub 2011/07/06. eng.

245. Rao R. Endotoxemia and gut barrier dysfunction in alcoholic liver disease. Hepatology (Baltimore, Md). 2009 Aug;50(2):638-44. PubMed PMID: 19575462. Epub 2009/07/04. eng.

246. Brenchley JM, Price DA, Schacker TW, Asher TE, Silvestri G, Rao S, et al. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. Nature medicine. 2006 Dec;12(12):1365-71. PubMed PMID: 17115046. Epub 2006/11/23. eng.

247. Bottcher K, Rombouts K, Saffioti F, Roccarina D, Rosselli M, Hall A, et al. MAIT cells are chronically activated in patients with autoimmune liver disease and promote

pro-fibrogenic hepatic stellate cell activation. Hepatology (Baltimore, Md). 2018 Jan 12. PubMed PMID: 29328499.

248. Komanduri S, Thosani N, Abu Dayyeh BK, Aslanian HR, Enestvedt BK, Manfredi M, et al. Cholangiopancreatoscopy. Gastrointestinal endoscopy.84(2):209-21.

249. Kurioka A, Jahun AS, Hannaway RF, Walker LJ, Fergusson JR, Sverremark-Ekstrom E, et al. Shared and Distinct Phenotypes and Functions of Human CD161++ Valpha7.2+ T Cell Subsets. Frontiers in immunology. 2017;8:1031. PubMed PMID: 28912775. Pubmed Central PMCID: PMC5582200. Epub 2017/09/16. eng.

250. Reantragoon R, Corbett AJ, Sakala IG, Gherardin NA, Furness JB, Chen Z, et al. Antigen-loaded MR1 tetramers define T cell receptor heterogeneity in mucosal-associated invariant T cells. The Journal of experimental medicine. 2013 Oct 21;210(11):2305-20. PubMed PMID: 24101382. Pubmed Central PMCID: PMC3804952. Epub 2013/10/09. eng.